Abstracts for the "Congrès Annuel de Recherche Dermatologique 97"

Sponsored by the "Société de Recherche Dermatologique" Faculté de Médecine de Tours—France, April 10–11, 1997

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0022-202X/97/$10.50 •

2C

Primary sensitization of naive T cells to haptens by Langerhans dendritic cells generated from cord blood $CD34^+$ precursors. N. Rougier, M.J. Gariazzo, G. Redziniak, D. Schmitt, C. Vincent. INSERM U346, Pavillon R, Hôpital E. Herriot, Lyon, France.

Langerhans cells (LC) play a key role in contact hypersensitivity reactions. The application of haptens on the skin lead to many modifications of these cells including the increase of MHC II expression, the increase of allogeneic stimulation potency and migration towards lymph nodes to activate T cells. Morever, it was shown that LC cultivated *in vitro* were able to induce a primary immune response to haptens. We generate from cord blood $CD34^+$ precursors the dendritic cells with caracteristics of LC (DCL) which represent only a part of the dendritic cells (DC) obtained. We show that these cells were able to sensitize syngeneic T cells to haptens like TNP and FITC. The response to TNP is higher than to FITC whereas SDS, an irritant molecule used as control, is never efficient. Phenotypic analysis of cellular suspensions and experiments of cell sorting show that only $CD1a^+$ cells were able to induce a stimulation of syngeneic T cells to TNP or to FITC. Furthermore, we show a relationship between differenciation state of DC and their ability to stimulate T lymphocytes. These cells were able to present haptens in efficient fashion during a period comprised between day 10 and day 14. Before day 10, this function is limited despite the presence of functional HLA-DR in MLR and the presence of efficient accessory molecules in mitogenic tests. This results allowed the hypothesis of processing linked to maturation of cells.

3C

CD101, A MAJOR ANTIGEN FOR THE ACTIVATION OF T LYMPHOCYTES BY SKIN DENDRITIC CELLS, IS IDENTICAL TO THE V.7 ANTIGEN. M. Bagot, K. Hall*, G.J. Freeman*, L. Boumsell, A. Bensussan. Inserm U448, Hôpital Henri Mondor, Créteil, France and *Dana-Farber Cancer Institute, Boston, MA, USA.

CD101 is a transmembrane antigen defined by two monoclonal antibodies raised in our laboratory BB27 and BA27. We have shown that CD101 is found on a major subpopulation of skin dendritic cells expressing the following antigens: HLA-DR, CD1a, CD1c, CD11a, CD11c, CD40, CD50, CD54, CD58, CD80, CD83, and CD86. CD101 is a 140 kD homodimer expressed by skin dendritic cells, cells of the monocytes/macrophages lineage, a subpopulation of activated T lymphocytes, and the most intestinal T lymphocytes. We used COS cell expression cloning to isolate a 4.2 kB cDNA encoding CD101. COS or Jurkatt cells transfected with the CD101 cDNA were recognized by the anti-CD101 monoclonal antibody BB27. DNA sequencing of the CD101 cDNA showed that CD101 was extremely similar to the recently identified T cell antigen V7. The CD101 cDNA sequence differed in the transmembrane region and the 3' region. The monoclonal antibody , specific for the V7 protein, recognized the CD101 transfectants. The CD101/V7 gene encodes a transmembrane protein containing seven immunoglobulin type Ig-V domains in the extracellular domain. Cross-block experiments showed that BB27 and V7.1 recognize distinct epitopes of the CD101 molecule. BB27 and V7.1 inhibit the activation of T lymphocytes by skin dendritic cells, both in allogeneic reactions and in soluble antigen specific reactions. This inhibition is overcome by addition of high doses of exogenous IL-2. CD101 could thus be a new costimulation molecule, which ligand remains to be determined.

4C

RELATIVE CONTRIBUTIONS OF EPIDERMAL AND DERMAL DERIVED ANTIGEN PRESENTING CELLS TO THE INDUCTION OF CONTACT HYPERSENSITIVITY. A. Bouloc and S.I. Katz. Dermatology Branch, NCI, Bethesda, MD, USA.

During contact sensitization, allergen is transported from skin to regional lymph nodes by cutaneous antigen presenting cells (APC). In this study, we determined the relative contribution of epidermal- and dermal- derived APC to the induction of contact sensitivity. Ear skin was removed from BALB/c mice 1 hour after painting with 20 µl of 1% trinitrochlorobenzene (TNCB), and whole skin (ws) or dispase-separated epidermis (e) or dermis (d) were placed in organ culture. After 3 days, migrating APC were recovered and characterized. wsAPC and eAPC reproducibly stimulated proliferation of in vivo primed T cells ($5x10^3$ APC + $2x10^5$ T cells = 25,589 cpm for wsAPC and 20,379 cpm for eAPC) whereas equal numbers of dAPC from painted skin and equal numbers of migrating cells from non painted skin did not induce T cell proliferation above background. In addition, $5x10^4$ eAPC from TNCB-painted skin sensitized naive mice (hapten-induced ear swelling 14.2 +/- $1.7x10^{-2}$ mm vs 3.5 +/- $1.4x10^{-2}$mm with eAPC from normal skin), while dAPC from TNCB-painted skin did not sensitize naive mice. wsAPC, eAPC and dAPC presented antigen to primed T cells after TNCB modification in vitro equally well, indicating that dAPC were active. Taken together, these data indicate that epidermal-derived, compared to dermal-derived, APC are more potent sensitizers after in vivo exposure to contact allergens.

7C

Vascular permeability factor (VPF/VEGF) release by human keratinocytes is up-regulated by contact allergens and down-regulated by hydrocortisone. S Palacio, J.Viac, D. Schmitt. INSERM U346, Hôpital Ed Herriot, 69437 Lyon 03.

In allergic contact dermatitis, keratinocytes are major target cells that can be activated to participate to the local reaction especially through the secretion of soluble mediators. Among the growth factors produced by keratinocytes, vascular endothelial growth factor (VEGF) is a powerful inducer of permeability of endothelial cells, involved in inflammation. We determined whether different contact allergens, dinitrosulfobenzene (DNSB), para-phenylenediamine (pPD) and the metals nickel and chromium in comparison to cobalt, described to mimic hypoxia, can modify the basal level of VEGF of normal human keratinocytes when tested at various, non-toxic concentrations. The effects of hydrocortisone were also tested. Our results showed an intense dose-dependent up-regulation of VEGF release by keratinocytes after treatments by metals and pPD. DNSB induced only a moderate increase of VEGF. Hydrocortisone reduced the basal level as well as the nickel-induced up-regulation of VEGF. These findings suggest that contact allergens up-regulate VEGF expression in keratinocytes likely by different mechanisms and may directly contribute to the microvascular hyperpermeability which characterize contact dermatitis whereas hydrocortisone drastically reduces this expression.

8C

EFFECTS OF CD40 LIGATION ON NORMAL HUMAN KERATINOCYTE ACCESSORY FUNCTION. J.Grousson, J.Péguet-Navarro, D.Schmitt. INSERM U 346, Lyon, France.

Interactions of CD40 on antigen-presenting cells with its ligand on activated T cells are known to play a key role in cellular, as well as humoral, immune responses. In a previous study, we have demonstrated the expression of CD40 on normal human keratinocytes, especially on the basal cell layer. Here, we analyzed wether CD40 ligation on these cells could alter their phenotype and their accessory function. To this end, normal human keratinocytes were grown for 2-4 days in DMEM medium supplemented, or not, with interferon-γ (IFNγ) using CD40-ligand transfected cells (CD40Lc), or control CD32 transfected cells (CD32c), as feeders. Keratinocytes were then recovered and processed for phenotypical and functional assays. Results showed that, in the absence of IFNγ, CD40 ligation on normal human keratinocytes did not induce ICAM-1, HLA-DR, B7-1, nor B7-2 at the cell surface. In the presence of IFNγ, CD40 triggering did not alter the expression of B7 molecules but it resulted in enhanced ICAM-1 and decreased HLA-DR expression on keratinocytes. We found that prior ligation in the presence, or not, of IFNγ did not alter the capacity of normal human keratinocytes to mount PHA or SEB dependent T cell proliferation. Furthermore, keratinocytes grown on CD32c, or on CD40Lc as well, were quite unable to induce primary allogeneic T cell reaction. Very interestingly, however, CD40 ligation on IFNγ-treated keratinocytes results in an enhanced autologous T cell response. Collectively, these results show that CD40 is functional on normal human keratinocytes and that it may play a role in some skin pathologies characterized by a T cell epidermal infiltrate, such as psoriasis and cutaneous T cell lymphomas.

9C

PRODUCTION OF IMMUNOREGULATORY CYTOKINES BY HUMAN EOSINOPHILS E. Lengrand*‡, E. Delaporte*, G. Woerly‡, M. Capron‡

* Service de Dermatologie A, CHRU, Lille. ‡ Unité INSERM U167, Institut Pasteur, Lille, France.

Many dermatological diseases are associated with an increased number of eosonophils in blood or tissues, which seems to be involved in the development and the persistence of the lesions as in atopic dermatitis or bullous pemphigoid. In order to show a possible selective secretion of type 1 or type 2 cytokines we studied the expression of immunoregulatory cytokines by human eosinophils. Peripheral blood eosinophils were purified by negative selection using the MACS system. After permeabilization by saponin, they were incubated with specific monoclonal antibodies against IFNγ, IL-2, IL-10 or IL-4, and analyzed by flow cytometry. Our results showed that IL-2, IFNγ, and IL-10 were strongly detected in more than 95% of the eosinophils isolated from healthy donors (n=3), or hypereosinophilic patients [bullous pemphigoid (n=4), atopic dermatitis (n=6), HES (n=3)], suggesting that these three cytokines are constitutively produced. On the other hand, only a small proportion of the cells expressed IL-4 (33% ±27, Mean Fluorescence Intensity=20). Similar results were found by immunocytochemistry. After 18 hours of culture, a second peak of fluorescence was observed (40% ±9, MFI=44), indicating that eosinophils are able to newly synthesize IL-4. To confirm this hypothesis, we have used an inhibitor of protein secretion, brefeldin A, in our culture system. No changes in the expression of IL-2, INFγ, and IL-10 were seen, whereas a strong increase of IL-4 expression was detected (67% ±22, MFI=90), showing that this cytokine accumulates upon brefeldin A into eosinophils . Taken together these results suggest that eosinophils can produce and secrete IL-4, whereas IL-2, IFNγ and IL-10 are stored but not secreted. The fact that eosinophils are able to selectively secrete IL-4, let us to speculate that they could be involved in the regulation of immune response and the development of diseases characterized by Th 2 responses .

*Abstract nos. 1C, 5C, and 6C are unpublished.

11C

RETINOIC ACID RECEPTORS (RAR) α AND γ AND GLANDULAR METAPLASIA IN MOUSE SKIN. S. Blanchet, G. Chevalier, P Kastner*, J.J. Michaille and D. Dhouailly. Biologie de la Différenciation Epithéliale, LEDAC, UMR-CNRS 5538, Institut A. Bonniot, La Tronche, Grenoble. *Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/Université Louis Pasteur, B.P. 163, 67404 Illkirch Cedex, C.U. de Strasbourg.

Retinoic acid (RA) treatment of embryonic mouse upper-lip skin leads to the development of eccrin glands instead of hair vibrissa follicules. This occurs at a concentration of RA ($1.67.10^{-5}$ M) that is sufficient for indirect activation of RXR. The formation of glands after treatment with an agonist for the three RARs (Ro 13-7410; 10^{-8}M) confirms that these receptors are directly involved. We therefore studied the potential involvement of two RARs, α et γ, known to be expressed during embryonic skin morphogenesis, by using RAR α and γ knock-out mice and RAR α and γ specific agonists.

The upper-lip skin was dissected from 12.5 and 13.5 day embryos. The explants were cultured for 48h on a grid in a dish containing DMEM, 20% FCS and a retinoid first disolved in DMSO or in the solvent alone.The explants were then grafted onto nude mice kidneys and allowed to develop for 9 days prior to analysis. The formation of gland in the explants which were treated either with a RARα (RO 40-6055, 8.10^{-9} M) or a RARγ (CD 437, $7.7.10^{-8}$ M) specific agonist suggests that both RAR could be involved. Further support is provided by the finding that both RARα -/- and RARγ -/- embryonic skin explants are capable of forming glomerular glands instead of hair vibrissa follicles after RA treatment. However, the mean-number of glands per explant was half less for RARγ -/- (6) than for the RARα -/- (12) explants.

In conclusion, our results confirm the involvement RARγ in mouse skin morphogenesis and show a partial redundance with the RAR α.

12C

THROMBOMODULIN, A FUNCTIONAL SURFACE PROTEIN ON HUMAN KERATINOCYTES, IS REGULATED BY RETINOIC ACID. P Senet, N Peyri, M Berard, L Dubertret, MC Boffa. Unité INSERM 353 et service de Dermatologie, Hôpital Saint Louis, Paris, France.

Thrombomodulin, a major anticoagulant proteoglycan of the endothelial cell membrane, is a thrombin receptor that acts as a cofactor for protein C activation. It was previously shown that thrombomodulin, present in human epidermis and in lysates of cultured keratinocytes was implicated in cellular differentiation during mouse fetal development. The role of retinoic acid in keratinocyte differentiation prompted us to study retinoic acid regulation of thrombomodulin expression in primary cultures of keratinocytes isolated from adult human skin, grown at low (undifferentiated keratinocytes) and normal calcium levels (differentiated keratinocytes). Thrombomodulin antigen level and total and surface activities were measured in cultures without and with retinoic acid. Thrombomodulin mRNA visualized by *in situ* hybridization was quantified by computer-based image analysis. Functional thrombomodulin was expressed on the surface and in the cytoplasm of cultured human keratinocytes regardless of the calcium concentration. In contrast, retinoic acid induced significant rises of the total antigen level and of surface and intracellular thrombomodulin activities only in keratinocytes grown in a low-calcium medium. In these undifferentiated keratinocytes, quantification of mRNA transcripts showed a 3-fold increase after retinoic acid stimulation. Thus, functional thrombomodulin is a human keratinocyte surface protein whose expression is controlled through the keratinocyte's differentiation program and is modulated *in vitro* by retinoic acid.

(accepted for publication in Archives of Dermatological Research)

13C

EFFECTS OF CD 2665, A SELECTIVE RARβ, γ ANTAGONIST ON THE RETINOID ACTIVITY IN HUMAN KERATINOCYTES AND FIBROBLASTS IN CULTURE. P. Ancian, M. C. Lenoir and S. Michel. *In Vitro* Studies, CIRD GALDERMA, Sophia Antipolis, 06560 Valbonne, France.

The effects of retinoids on the differentiation of normal human keratinocytes in culture were investigated by measuring the expression level of the cross-linking enzyme transglutaminase type 1 (TG1). CD 367, a potent agonist of the three retinoic acid receptors RARα, β and γ inhibits in dose-dependent manner the expression of this differentiation marker. CD 2665, a selective antagonist of the RARβ, γ pathway has no effect when applied alone but inhibits completely the down regulation of the expression of TG1 induced by CD 367.

The retinoid activity was also determined in human dermal fibroblast by determining the mRNA contents of CRABP II, RARβ and ICAM-1 using semi-quantitative RT-PCR. CD 367 produced a dose-dependent up-regulation of the transcription of these three markers. CD 2665 alone did not produce any effect but inhibited the activity of CD 367 on the expression of these mRNAs.

The RAR content in these two cell types was determined by RT-PCR experiments. Normal human keratinocytes express both RARα and γ, while dermal fibroblasts contain the three RAR subtypes. These results show that the retinoid activity in these cells is probably mediated by RARγ (and/or RARβ in fibroblasts), but not by RARα. To confirm this hypothesis, experiments with a selective RARα antagonist are in progress.

15C

EXPRESSION OF PERIPHERAL BENZODIAZEPINE RECEPTORS IN HUMAN SKIN. P.E. Stoebner, P. Carayon*, G. Penarier*, N Frechin*, G. Barneon, P. Casellas*, D Dussossoy*, JP Cano**, J. Meynadier, L. Meunier. Service de Dermatologie, SANOFI Recherche*, Laboratoire de Toxicologie du Médicament**, Montpellier, France.

Peripheral benzodiazepine receptors (PBR) are important regulatory sites of the cellular metabolism. Recent data indicate that they may prevent hematopoietic cell lines from oxygen radical damage. We studied the tissular and cellular repartition of PBRs in normal human skin by using a specific monoclonal antibody (8D7) directed against the C-terminal fragment of the receptor. PBRs are present in keratinocytes (Kc), Langerhans cells (LC) and pilosebaceous follicles. The epidermal receptor expression was consistently stronger in superficial differentiated layers and cytometric quantification of intracytoplasmic PBRs in human epidermal cell (EC) suspensions allowed us to determine the mean number of antigenic sites per cell in LC ($531 \pm 49 \times 10^3$) (n=3), Kc with low ($474 \pm 41 \times 10^3$) and high ($1095 \pm 14 \times 10^3$) granularity. Confocal and electron microscopic examination revealed that epidermal PBRs were exclusively localized on the mitochondrial outer membrane. The increased PBR expresion in differentiated EC was not associated with an increased number of mitochondria per cell. By using in situ hybridization, the expression of mRNA correlates with that of protein. In human dermis, PBR are present in fibroblasts, nerves and endothelial cells. The expression of PBRs was similar in UV-exposed skin (n=3), melanomas (n=3) and carcinomas (n=6). These results suggest an important role of PBRs for human epidermal homeostasis.

16C

CUTANEOUS CYP2E1 INDUCTION AFTER TOPICAL TREATMENT BY DEXAMETHASONE, ETHANOL AND SALICYLIC ACID IN MOUSE. E. Sampol, A. Mirrione, PH. Villard, E. Cauture, H. Scoma, P. Berbis, B. Lacarelle, A. Durand. EA 2194 and URA- CNRS 1924, Faculté de pharmacie Marseille

Expression and inducibility of cutaneous cytochromes P450 remain largely unknown. We studied CYP2E1 (a known isoform which activates procarcinogens) and CYP3A subfamily expressions in liver and skin microsomes from mouse treated topically for four days by dexamethasone (an inducer of hepatic CYP3A), ethanol (an inducer of hepatic CYP2E1), and salicylic acid (an inducer of hepatic CYP2E1). With the aim to study the influence of the route of administration, dexamethasone was also administrated intraperitoneally, daily, for four days. Paranitrophenol-hydroxylase and erythromycine-N-demethylase (ERD) enzymatic activities were determined. Cutaneous and hepatic expression of CYP2E1, CYP3A and their mRNA were estimated respectively by western blot and RT-PCR analysis.

Results showed an increase in ERD hepatic activity after dexamethasone treatment. Hepatic CYP3A expression was increased by topical and intraperitoneal administration of dexamethasone. Hepatic CYP2E1 expression was not modified by the tested compound. Cutaneous CYP2E1 expression was increased by topical application of dexamethasone (8 fold), ethanol (4 fold), and salicylic acid (3 fold). Induction of cutaneous CYP2E1 after intraperitoneal treatment by dexamethasone was lower (4 fold). Cutaneous CYP2E1 mRNA was only increased after dexamethasone treatment. CYP3A and its mRNA were never detected in skin microsomes.

In conclusion, we observed that the induction process can be different between tissues. Tissue specific induction was observed for dexamethasone (induction of hepatic CYP3A and of cutaneous CYP2E1). Induction mechanisms of cutaneous CYP2E1 could be different depending on the tested compounds.

17C

ACQUISITION OF A SLOW ACETYLATOR PHENOTYPE IN AIDS PATIENTS WITH A RAPID ACETYLATOR GENOTYPE: AN EXPLANATION FOR HIGH RATE OF CUTANEOUS SULFONAMIDES REACTIONS IN AIDS POPULATION? P. Wolkenstein[1] F. Baud-Camus[1], S. Lecoeur[2], JD Poveda[3], and the Epitox Group.

[1]Dermatology, CHU Henri-Mondor, Créteil; [2]Inserm 75, CHU Necker, [3]Virology, Institut Pasteur, Paris, France

Sulfonamide-induced reactions have been linked with a slow acetylator phenotype. The slow acetylator genotype is a risk factor for sulfonamide-induced toxic epidermal necrolysis. In AIDS patients, an unexpected high rate of slow acetylator phenotype has been found. We demonstrated the acquisition of a slow acetylator phenotype in AIDS patients with a rapid acetylator genotype during acute illnesses.

15 AIDS patients hospitalised for pneumocystosis or toxoplasmosis and included in a study of cutaneous drug reactions in AIDS (Epitox study) were evaluated for acetylation. Patients DNA was amplified by PCR and tested for N-acetyltransferase 2 mutations M1, M2, M3, M4. Individuals were considered to have a slow acetylator genotype if 2 of the 4 mutations were present and to have a rapid acetylator genotype if no or only one mutation was present. After admission, patients were given caffeine (200 mg). Urines samples were collected 4 hours later. Urinary concentrations of 5-acetylamino-6-amino-3-methyluracil (AAMU), and 1-methylxanthine (1X) were measured. The ratio AAMU/1X assessed the phenotype. Patients with ratio < 1.8 had a slow acetylator phenotype. Patients with ratio ≥ 1.8 had a rapid acetylator phenotype. 5 patients had a slow genotype, 10 a rapid genotype.9 had a slow phenotype, and 6 a rapid phenotype. 4 patients with a rapid genotype expressed a slow phenotype.

Acute illnesses in AIDS patients can modify the expression of a rapid acetylator genotype into a slow acetylator phenotype. This modification of acetylation expression could explain the high rate of cutaneous sulfonamides adverse reactions in this population.

*Abstract nos. 10C and 14C are unpublished.

18C

Diffusion of fusidic acid (FA), oxacillin (O) and pristinamycin (P) in interstitial dermal fluid after repeated oral administration. L. Vaillant, Ch. Leguellec, R. Barruet, F. Jehl, C. Renault-Gallion, H. Sorensen, E. Autret, R. Roiron, G. Lorette (Tours, Strasbourg, Paris).

This cross-over randomized study was carried out in 12 healthy male volunteers. The mean age was 24,5 plus ou moins 3,9 years and weight 67,8 plus ou moins 7,6 kg. The antibiotics were given twice a day for 5 consecutive days : **FA**, 1 g/d, **O** and **P** 2 g/d. Suction blisters were produced by application of negative pressure (-300 mmHg) for 2 hours on the volar aspect of the forearm. Serum and interstitial fluid (IF) samples were taken 0, 2, 4, 6, 8, and 12 hours after the last oral administration at day 6, and the dosages carried out by HPLC. The main results are the following ones :

	SERUM			LBS			LBS/Sérum	
	Cmax mg/l	Tmax h	AUCss mgh/l	Cmax mg/l	Tmax h	Cmin mg/l	AUCss mg.h/l	Cmax %	AUCss %
AF	90,3±23	2,6±1,2	815±251	44,4±15	6,2±1,3	31,6±12,4	467±183	48±8	57±13
O	8,3±3,6	1,1±0,3	13,8±5,4	1,0±0,5	2,8±1,0	0,10±0,13	6,3±3	13±5	48±21
P	0,5±0,4	1,4±0,7	<L	0,3±0,2	2,6±1,3	0,02±0,04	1,3±1,8	73±57	114±61

Diffusion (%) is higher for **P** than for **FA** and **O**. However with regards to serum and IF inhibitory quotients (MICs) for susceptible *Staphylococcus aureus* : **FA**, 0,04 to 4 ; **O** : 0,25 to 0,5 ; **P** : 0,2 to 0,3 mg ; **FFA**compares very favourably with **O**and **P**.

19C

THE VHCDR3 OF A HUMAN ANTI-DESMOSOMAL PLAQUE ANTIBODY SHARES AN AMINO-ACID SEQUENCE WITH THE DESMOGLEIN I CYTOPLASMIC DOMAIN INTERACTING WITH THE PLAQUE. A NEW SELF-ANTIGEN BINDING MECHANISM OF AUTOANTIBODIES ?

Gilbert D, Courville Ph, Brard F, Joly P, Delpech A, Lauret P, Tron F. Laboratoire d'Immunopathologie, Clinique Dermatologique, GRIMP, (IFR23) CHU Charles Nicolle, 76031 Rouen, France.

Pemphigus are autoimmune bullous skin diseases caused by autoantibodies directed against components of the desmosomal junction. F12 is a monoclonal antibody derived from a patient with pemphigus vulgaris. It was previously shown to bind to a 185-kD polypeptide by immunoblot using bovine tongue epithelium extract as the substrate and to recognize a component of the desmosomal plaque by immunoelectron microscopy. We have cloned and sequenced the heavy and light chain variable region genes of mAb F12 and showed that the CDR3 of the heavy chain shared a 4 aminoacid sequence (Glycine-serine-serine-Glycine) with the intracellular domain of desmoglein I, likely involved in the interaction with components of the desmosomal plaque. Computer modeling of F12 showed that the GSSG sequence might contribute to antigen interactions. The GSSG sequence was further demonstrated to be essential to F12 function since a peptide encompassing the VHCDR3 and a GSSG tetrapeptide inhibited its binding to target antigens while VH-CDR3 peptides with specific modifications of the GSSG sequences did not. Since mAb F12 reacts with the desmosomal plaque, it is likely that its antigen-binding site mimicks the glycine- and serine-rich cytoplasmic binding domain of desmoglein I. This observation suggests that some autoantibodies produced in the course of pemphigus might behave as adhesion molecules and, thus a new self-antigen binding mechanism of autoantibodies.

20C

PRODUCTION OF ANTI-IDIOTYPE ANTIBODIES DIRECTED AGAINST ANTI-190-kD ANTIBODIES IN PEMPHIGUS. C. Richard, D. Gilbert, A. Delpech, Ph. Lauret., P. Joly, F. Tron. *Groupe de Recherche en Immunopathologie (GRIMP) Hôpital Ch. Nicolle - Rouen.*

Introduction : MAb F12 is a human mAb directed against a 190-kD antigen of the desmosomal plaque which seems to be representative of a new autoantibody population present in the different types of pemphigus.

Materials and Methods: Mices were immunized with mAb F12. Murine hybridomas were then generated and the supernatants were screened by ELISA in order to distinguish between anti-isotype and anti-idiotype antibodies. Anti-idiotype murine mAbs were analyzed by indirect immunofluorescence and immunoblotting using bovine tongue extracts as the substrate.

Results : 6 hybridomas producing anti-F12 idiotype antibodies were obtained. All these anti-F12 idiotype antibodies identified mAb F12 and not an irrelevant IgM human mAb. Moreover, 3 anti-F12 idiotype mAbs inhibited the binding of mAb F12 to the 190-kD protein, indicating that they were directed against paratopic sites of mAb F12. Finaly, some anti-F12 idiotype mAbs identified anti-190-kD antibodies present in 2 of the 6 pemphigus sera tested.

Discussion: Anti-idiotype antibodies which are directed against recurrent idiotypes, can identified some autoantibody populations produced in autoimmune diseases. To date, no anti-idiotype antibodies have been produced in pemphigus. The anti-F12 idiotype antibodies obtained in this study confirmed that mAb F12 is representative of a new autoantibody population present in the different types of pemphigus.

21C

THE ANTIGENIC SPECIFICITIES RECOGNIZED BY IgG4 ANTIBODIES DETERMINE THE TYPE OF AUTOIMMUNE BULLOUS SKIN DISEASE IN PATIENTS WITH MULTIPLE AUTOANTIBODIES DIRECTED AGAINST BOTH DESMOSOMES AND THE BASEMENT MEMBRANE ZONE. P. Joly, D. Gilbert, E. Thomine, C. Le Corvaisier-Piéto, A. Delpech, Ph. Lauret, F. Tron. Groupe de Recherche en Immunopathologie (GRIMP) Rouen.

Design : To identify factors which determine the clinical and histologic features of autoimmune bullous skin diseases (BSD) in patients with multiple autoantibodies directed against components of desmosomes and the basement membrane zone (BMZ).

Patients and methods : 5 patients with BSD whose sera recognized both the epithelial cell surface (ECS) and the BMZ by indirect IF were included. Sera were analyzed by indirect immunoelectronmicroscopy (IEM) and by immunoblotting using human epidermal and bovine tongue extracts as the substrates and were then revealed by anti-human Ig subclass murine mAbs.

Results : IEM analysis of the 5 sera showed that they labeled both desmosomes and the BMZ. The table shows the antigenic specificities recognized by each IgG subclass of these sera.

Patients	Type of disease	DIF	IgG 1	IgG 2	IgG 3	IgG 4
1	BP	BMZ	BPAG2 - DSG1	BPAG2	BPAG2 - DSG1	BPAG 2
2	BP	BMZ	BPAG1 - BPAG2	DSG1	-	BPAG 2
3	BP	BMZ	DPK1-2 - BPAG2	BPAG2	-	BPAG 2
4	PV	ECS	BPAG1	-	-	DSG 3
5	PF	ECS	BPAG1	-	-	DSG 1

The clinical and histologic features and DIF pattern of patients 1 to 3 were typical of bullous pemphigoid (BP) and those of patients 5 and 6 were typical of pemphigus vulgaris (PV) and pemphigus foliaceus (PF) respectively.

Discussion : The clinical and histologic features of the 5 patients seem to be determined by the antigenic specificities recognized by circulating IgG4 autoantibodies.

22C

BOTH EPITOPES RECOGNIZED BY THE AUTOANTIBODIES OF CICATRICIAL PEMPHIGOID ON BP180 ARE PRESENT IN THE TARGET TISSUES OF THIS DISEASE. F. Caux,*# M. Olague-Marchan,* S.D. Balding,* P.A. Rose,* C. Prost,# D. Zillikens,* L. A. Diaz,* G.J. Giudice* *Departments of Biochemistry and Dermatology, Medical College of Wisconsin, Milwaukee, U.S.A., #Hôpital Saint-Louis, Paris, France.

Cicatricial pemphigoid (CP) is a bullous disease that predominantly involves mucous membranes. Although the target antigen in CP is still under discussion, BP180 has been demonstrated to be the major target antigen of the autoantibodies and two distinct epitopes, one in the carboxy-terminal region and the other in the non-collagenous 16A domain of BP180 have been identified. To have more evidence that BP180 could be involved in the pathophysiology of CP, we checked the presence of these epitopes in the human target tissues of CP, i.e. gum, tongue, palate, conjunctiva, vocal chord, vagina, oesophagus, trachea, and buccal, nasal and genital mucosae. For this aim, we generated rabbit polyclonal antibodies directed to both epitopes. We used these antibodies to detect these epitopes by indirect immunofluorescence (IF) and immunoblotting. The results of our investigation demonstrated that both epitopes are present the target tissues of CP by indirect IF. At the protein level, an antigen was detected in all tissues except conjunctiva and had the same molecular weight than in epidermis. Several primers specific for epidermal BP180 hybridized total RNA from buccal mucosa and conjunctiva. A complete homology of a 734 bp sequence including the sequence of the carboxy-terminal epitope of epidermal BP180 was found by sequence analysis of the reverse transcriptase-polymerase chain reaction amplification products from buccal mucosa and conjunctiva. In conclusion, two epitopes identified in CP on epidermal BP180 are present in the target tissues of CP and may be involved in the genesis of the mucosal lesions.

23C

ADHESION AND APOPTOSIS MOLECULES EXPRESSION IN CUTANEOUS ADVERSE DRUG REACTIONS INDUCED BY ANTIBIOTICS. Barbaud A*, Béné M-C°, Gobert B°, Jacquin-Petit M-A*, Schmutz J-L*, Faure G°. *Service de Dermatologie, Hôpital Fournier ; °Laboratoire d'Immunologie, Faculté de Médecine & CHU, Nancy, France.

Cellular immunophenotyping on skin biopsies, useful to identify immunological mechanisms involved in cutaneous adverse drug reactions (CADR) was applied to 16 CADR with a very probable or probable responsible drug (1 fixed drug eruption to cefodoxime, 1 acute generalized exanthematous pustulosis to penicilline M, 4 maculopapular rashes (MPR) to amoxicillin, 1 MPR to isoniazide, 1 MPR to norfloxacin, 3 MPR and 4 erythrodermias to pristinamycin and 1 urticaria to spiramycin). Indirect immunofluorescence phenotyping was performed on skin biopsies with monoclonal antibodies to CD1, HLA DR, CD3, CD4, CD8, CD25, CD11a/CD18, CD54, CD106, CD62E, CD62L, CD62P, CD95 and bcl2. Normal numbers of CD1+ Langerhans cells were observed, 20 % of which were also HLA DR+. Dermal CD1+ dendritic cells were present in 9/16 cases (all the CADR due to betalactams and 6/9 MPR). Perivascular T lymphocytes were CD3+CD4+. CD4+ or CD8+ cells were present along the DEJ in 7 cases especially in CADR induced by pristinamycin. T-cells were always LFA1+, and CD62L+ in 7 cases. Except in urticaria, endothelial cells were ICAM1+, CD62P+ in 13 cases and CD62E+ in 9. Activated keratinocytes were ICAM1+ in 11 cases (7MPR), HLA DR+ in 4 cases, CD62L+ in 3 erythrodermias, 1 MPR due to pristinamycin and in 1 MPR to isoniazide. After precipitation and Western Blot analysis the MW of this molecule was 76kD, similar to that of leucocytic CD62L. Keratinocytes expressed moderately CD95 in 7 cases and were bcl2+ in 4 cases (3 due to pristinamycin and 1 to isoniazide). Immunophenotyping data vary depending on clinical aspects and the responsible drug to the CADR. Here it confirmed that delayed cellular hypersensitivity are involved in all the CADR studied but urticaria.

24C

RESTRICTED T-CELL Vß REPERTOIRE DIVERSITY IN PERIPHERAL BLOOD AND TISSUE INFILTRATING LYMPHOCYTES IN OMENN'S SYNDROME (SEVERE COMBINED IMMUNODEFICIENCY WITH ERYTHRODERMA AND HYPEREOSINOPHILIA)

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Omenn's syndrome is a rare inherited immunodeficiency characterized by early erythroderma, severe recurrent infections, protracted diarrhea, and marked hypereosinophilia. Contrasting with a severe T-cell depletion in lymphoid tissues, the number of circulating T-cells is elevated and a large number of T-cells also infiltrate the skin and the intestine, but these T-cells are poorly functional in vitro.

We analyzed T-cell Vß repertoire diversity in circulating and tissue infiltrating lymphocytes in an infant with Omenn's syndrome, with a large panel of monoclonal antibodies to 17 different variable segments of the T-cell receptor ß chain (TCR-Vß):Vß2, Vß3, Vß5.1, Vß5.2, Vß5.3, Vß6.1, Vß8, Vß9, Vß12, Vß13.1, Vß13.6, Vß14, Vß16, Vß17, Vß18, Vß20, and Vß21.

While all TCR-Vß segments are detected in circulating T-cells of normal infants, there was a restricted expression of only six different TCR-Vß segments (Vß3, Vß5.2, Vß9, Vß6.1, Vß12 and Vß14) in peripheral blood T-cells of the patient. PCR amplification and sequencing of the T-cell receptor ß chain confirmed that T-cell population was markedly oligoclonal. In the skin and in the intestine four of the previous TCR-Vß segments (Vß3, Vß5.2, Vß6.1 and Vß12) were found, but no additional TCR-Vß segment could be detected. In the skin Vß5.2 and Vß6.1 T-cells infiltrated the epidermis while Vß3 and Vß12 T-cells were almost exclusively located in the dermis.

These data suggest that in Omenn's syndrome, both circulating and tissular T-lymphocytes originate from an oligoclonal population, among which some clones could be reactive to epidermal antigens.

25C

ANTIBODIES DIRECTED AGAINST ENDOTHELIAL DERMAL CELLS DURING CUTANEOUS VASCULITIS. **V. Jan, H. Watier, L. Vaillant, G. Thibault, P. Bardos, G. Lorette. Department of Dermatology and Immunology, CHU Trousseau, C-37044 TOURS, FRANCE.**

Cutaneous vasculitis (CV) involve the agression of endothelium of dermal microvessels. Although their physiopathology is actually not well understood, some experimental models have been developped in the literature showing that CV could be mediated by immune complexes (IC). Antibodies or auto-antibodies directed against membrane antigens of endothelial cells (AECA) have also been described in sera from patients affected by different « immune-mediated » vascular damage, such as systemic vasculitis. However, their antigenic specificity is unknown and the prevalence is very variable.

In this study, we have investigated the presence of AECA in sera from 11 patients affected by CV, showing no systemic sign. Immunofluorescence assays were performed, followed by cytometry analysis. As compared to usual immunoenzymatic methods, flow cytometry was shown to be more sensitive and reproductible. Two cellular types were studied : human ombilical vein endothelial cells (HUVEC) and human dermal microvascular endothelial cells (HDMEC). Since endothelial cells are known to express class I HLA antigens, the 11 sera were previously screened for the presence of anti-HLA class I antibodies, using microlymphocytotoxicity assay.

With both HUVEC and HDMEC, 1 out of 11 sera displayed an anti-endothelial cell surface binding. This serum, extracted from a multipare patient, was found positive for anti-HLA class I antibodies. So, no AECA was detected in the sera from the 11 patients affected by CV. These results are in disagreement with the hypothesis that AECA are present during CV showing no systemic sign. Since anti-HLA antibodies detection is often not performed, the presence of AECA could be ascribed to the presence of anti-HLA antibodies in the patient sera.

26C

PHOTOPROTECTION AND UV-INDUCED IMMUNOSUPPRESSION : A STUDY OF DNCB CONTACT HYPERSENSITIVITY (CHS) ON 160 VOLUNTEERS.

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UVB exposure reduces immunization rates to epicutaneous antigens in humans. The purpose of our study was to evaluate the protective effect of SPF 15 sunscreen on the reduction of contact hypersensitivity (CHS) responses that occurs in humans after an acute solar-simulated irradiation. After initial dinitrochlorobenzene (DNCB) sensitization (30µg) on the buttock, the elicitation was performed on unirradiated upper inner arm skin with a panel of four different doses of DNCB (3, 6, 9 and 12µg). The skin fold thickness was determined to score the elicitation phase and the irritating effects of DNCB. For each site, the mean increase of skin thickness due to the irritating effects of DNCB was subtracted from the increase in skin thickness due to the elicitation response. Sensitization on UV-irradiated sites was performed 3 days after a 3 MED UV-exposure (Dermolum solar simulator and a SPF 15 sunscreen formulation, containing a combination of Eusolex 232, Uvinul N539, Parsol 1789 and Mexoryl SX, was applied on the buttock to be irradiated. Upon recruitment each of male volunteers (n = 160) was randomly assigned into one of 8 groups (Gr): sensitization and elicitation with (Gr A-UV, n = 20) and without UV exposure (Gr A, n = 20); elicitation with (Gr B-UV, n = 20) and without UV (Gr B, n = 20); sunscreen application prior to sensitization and elicitation with (Gr C-UV, n = 20) and without UV exposure (Gr C, n = 20); elicitation after sunscreen application on a buttock with (Gr D-UV, n = 20) and without UV exposure (Gr D, n = 20). The A-UV group had a reduced response rate to challenge doses of DNCB compared with Gr A ($p \leq 0.005$) and Gr C-UV ($p \leq 0.015$). Gr A, Gr C and Gr C-UV showed no significant differences in responses rate to any of the doses of DNCB tested. The mean increases in skin thickness due to challenge reactions were similar in control groups (Gr B, Gr B-UV, Gr D and Gr D-UV). In conclusion, combinations of sunscreens with intermediate SPF but effective UVA protection may adequately prevent the suppression of CHS induced by an acute solar-simulated irradiation.

27C

SCREENING FOR PUTATIVE HOT SPOT MUTATIONS IN NEUROFIBROMATOSIS TYPE 1.

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The neurofibromatosis type 1 (NF1) gene spans 350 kb of chromosome 17q11 and contains 60 exons. Point mutations, that are expected in almost 70% of the NF1 cases, are spread over the whole gene. Given the high mutation rate of the NF1 gene and its high GC-content, we aimed to design a strategy for mutation detection based on the exhaustive analysis of the most GC-rich coding regions.

We use PCR-denaturing gradient gel electrophoresis (DGGE) as a scanning method for the analysis of exons 16, 28, 29 and 49. Computer-designed experimental DGGE conditions allow to detect any subtle change in a given DNA sequence. The mutations detected are further characterized by DNA direct sequencing. Sixty unrelated patients have been included in this study.

Exons 28, 29, 49 and a part of exon 16 have been analysed. Five mutations have been identified in exon 29: R1748X (found in two unrelated patients) and R1849Q, located at CG sites; W1810X, located at a CTG site; two putative splice variants 5206-2 A->G and 5511 A->G. Another putative splice disturbing mutation has been characterized in exon 28. In addition, two new biallelic polymorphisms were identified in introns 28 and 29. No mutation has been detected in the last coding exon of the NF1 gene (exon 49).

In this study, the DGGE analysis of a single exon (exon 29), the most GC-rich one and which accounts for 4% of the coding sequence, has allowed to identify a mutation in 10% of the patients.

28C

ABSENCE OF DETECTION OF HHV-8 SEQUENCES IN EPITHELIAL AND VASCULAR NEOPLASMS

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A new herpes virus has recently been associated with Kaposi's sarcoma, body cavity based lymphoma and possibly to multicentric Castleman disease. The restriction of HHV-8 to a limited number of disease is a strong argument against widespread virus. However, controversial data have been published on HHV-8 detection in other vascular neoplasms and epithelial proliferations. We have investigated the presence of HHV-8 sequences in i) various vascular neoplasms: 9 angiosarcomas, 4 cases of angiolymphoid hyperplasia and 1 case of Kimura's disease. ii) 12 squamous cell carcinomas, 6 basal cell carcinomas and 2 actinic keratosis from 17 different immunosuppressed transplant patients. DNA was extracted from snap frozen specimens (vascular neoplasms) or paraffin wax-embedded tissues, previously fixed with formalin (epithelial proliferations). Ten wax-embedded KS specimens fixed in formalin (3 HIV-related KS, 3 classic non HIV-related KS, 4 immunosuppressed transplant patients) were tested in the same conditions. We used primers amplifying a 233 basepair product derived from ORF 26 with the following nested primers sets : outer 5'-TGTATTAGCTAACCCTTCTAGCG-3', 5'-CCCGCTTCAAATCGTTAGCGTGGGG3' ; inner 5'-AGCCGAAAGGATTCCACCAT-3',5'-TCCGTGTTGTCTACGTCCAG-3'. PCR using β-globin sequences was used as control.While a 233 bp product was found in all frozen KS specimens and in 8 out of the 10 paraffin wax embedded KS specimens, all epithelial and vascular tumors remained negative. Our data argue against the hypothesis that HHV8 is a widespread latent virus involved in proliferative lesions of immunosuppressed patients. Moreover, HHV-8 detection in vascular lesions is a strong argument for Kaposi's sarcoma diagnosis.

29C

DETECTION OF HUMAN HERPESVIRUS 8 IN HIV NEGATIVE PATIENTS WITH CUTANEOUS LYMPHOMA

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HHV-8 is associated with Kaposi's sarcoma and with some lymphoproliferative disorders such as Castleman's disease and primary effusion lymphoma. Because HHV-8 shares homology with EBV which has been implicated in the pathogenesis of cutaneous lymphoma, we search for the presence of HHV-8 DNA sequences in various lymphoproliferative disorders of the skin in HIV negative patients. Fourty-seven HIV-patients were enrolled in the study. The diagnoses were as followed: mycosis fongoïdes (n = 23); Sezary syndrome (n = 5); pleomorphic T-cell lymphoma (n = 2), anaplastic large T-cell lymphoma (n = 2), T-cell lymphoma associated with HTLV-I (n = 1); B-cell lymphoma (n = 8); large plaque parapsoriasis (n = 6). Biopsies were stored at -80°C until process. DNA was obtained after a classical extraction by phenol/chloroform. Specific primers were used for the detection of human ß-globin gene and HHV-8. For HHV-8, a nested PCR was performed. PCR products were analyzed by agarose gel electrophoresis and hybridized with specific internal digoxigenin -labelled probes. The amplification of ß-globin was positive in all samples suggeting the absence of major PCR inhibitors. HHV-8 DNA sequences could not be detected in samples analyzed excepted in one biopsy from a 87-year old woman with large plaque parapsoriasis. Our study do not suggest any direct implication of HHV-8 in the patogenesis of cutaneous lymphoma in HIV neagative patients.

30C

HUMORAL AND CELLULAR IMMUNE RESPONSES IN ANIMALS TO PAPILLOMAVIRUS RECOMBINANT CAPSID VACCINE AND AFTER DNA IMMUNIZATION WITH THE L1 MAJOR CAPSID GENE.

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The L1 major capsid proteins of Human Papillomavirus types 6, 11, 16, 18 and 45 were expressed in insect cells with recombinant baculoviruses. The virus-like particles (VLPs) produced reacted with monoclonal and polyclonal anti-HPV-L1 antibodies. Purified HPV 16 recombinant particles were used to develop an HPV candidate vaccine. HPV vaccine doses containing 0.1 to 20 µg of VLPs were used in mouse, rabbit and sheep immunization studies. The results show that the recombinant capsids induced a strong humoral response and a T-cell response with a Th1 profile associated with secretion of both interferon-γ and interleukin-2.

DNA immunization was also investigated in mice in order to induce an anti-HPV 16 L1-specific immune response. The HPV 16 L1 gene was cloned into the pcDNA3 vector under the control of the CMV immediate early promoter. Anti-HPV 16 L1 VLP antibodies were observed in sera of the DNA immunized mice, but with a lower titer than HPV 16 L1 VLP immunized mice. However, the cellular immune response was higher than that observed in mice immunized with VLPs without adjuvant.

31C

Identification of potentially immunogenic sequences of human papillomavirus 16 (HPV 16) E6 and E7 proteins in human.

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INTRODUCTION HPV16 is associated with cervical intraepithelial neoplasia (CIN), cervical carcinoma and vulvar intraepithelial neoplasia. HPV 16 codes for 2 early regulatory proteins E6 and E7, which are necessary to induce transformation of keratinocytes and which are expressed in all infected and/or tumor cells. These E6 and E7 proteins are good candidates to be used as targets for cytotoxic T lymphocytes (CTL). Antigenic peptides from E6 and E7 could be associated with MHC class I molecules on the surface of infected cells and could be recognized by CTLs. The objective of this work was to study cytotoxic immune responses against E6 and E7 proteins in the aim to develop an immunotherapy or a vaccine against HPV16.

MATERIAL AND METHODS Pool of peptides spanning the whole proteins E6 and E7 were used to stimulate in vitro PBMC from 4 healthy donors and 1 HPV16 infected patient (CIN III) in presence of interleukines. Anti-E6 and anti-E7 lines were tested by chromium release test for their ability to lyse autologous B-EBV target cells sensitized by peptides or infected by recombinant vaccinia virus for E6 and E7.

RESULTS Our results showed that 3 regions of E6: E6 45-67, E6 80-88 et E6 121-140 and 2 regions of E7: E7 35-55 et E7 60-74 were immunogenic and recognized by CTL. These CTL lysed also B-EBV infected by Vac-E6 and Vac-E7 respectively.

DISCUSSION We have in this study determined immunogenic regions from E6 and E7 recognized by CTL. Peptides from these sequences could be good candidates for vaccination or immunotherapy. In order to determine other epitopes for CTL, this study will be extended to additional donors and patients.

32C

GENETIC AND PROTEIN EXPRESSION OF MANGANESE SUPEROXIDE DISMUTASE (MnSOD) IN DIFFERENT MELANOMA CELL LINES.

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In recent studies, a decreased expression of MnSOD, an intracellular enzyme responsible for dismutation of anion superoxide, has been reported in some malignant cells whereas its gene may have a antiproliferative effect on melanoma cells.

We have studied the expression of MnSOD both at the genetic (DNA, mRNA) and protein level in three parenteral cell lines of human origin (M3Da,M1Dor,M4Be), as in derived clones (1C8,T1C3) and variants (7GP22,T1P26). All these cell lines were tumorigenic using the nude mice model. In these cell lines, MnSOD gene was searched by PCR using genomic DNA, MnSOD transcripts by RT-PCR using mRNA extracts with primers allowing amplification of MnSOD gene between residues 179 and 595. Protein expression of MnSOD was studied by immunofluorescence (monoclonal antibody anti-human MnSOD Bender®) on suspended cells fixed on slides after cytospin.

Our results show that all studied melanoma cell lines including clones and variant contained detectable amounts of DNA and mRNA specific for the MnSOD gene. In contrast, there was a variable expression of MnSOD at the protein level. As detected by immunofluorescence, MnSOD protein was expressed in only 2 parenteral cell lines (strongly in M3Da cells, weakly in M4Be cells), but never in clones and variant cell lines.

These preliminary results are globally in agreement with a deficit of MnSOD protein expression, which was very variable depending on the melanoma cell line. In our melanoma cell lines, this deficit remains to be quantified at the protein level, but also to the genetic or functional level, as to be correlated with the metastatic potential in vitro (newborn raccoon model)

33C

A NEW TYPE OF SPECIFIC GENE FUSION GENERATED BY THE t(17;22) ASSOCIATED TO DERMATOFIBROSARCOMA PROTUBERANS AND GIANT CELL FIBROBLASTOMA.

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Dermatofibrosarcoma protuberans (DP) is an uncommon low grade, slow growing and locally aggressive tumor of the deep dermis. Although authentic DP may also, but seldomly, occurs in children, a distinct entity, giant cell fibroblastoma (GCF), has been considered as a juvenile form of DP.

DP presents specific chromosomal abnormalities such as reciprocal translocations t(17;22)(q22;q13) and supernumerary ring chromosomes derived from the t(17;22). Our previous fluorescent in situ hybridization (FISH) studies showed that the breakpoint on ring chromosome 22 was telomeric to the *LIF* gene which is located on 22q12.

We utilized a DP tumor containing the t(17;22)(q22;q13) translocation to clone the breakpoint. The translocation breakpoint was framed by systematic FISH analysis using cDNA, cosmid, BAC and YAC probes located between D22S411E and *BZRP* on 22q12-13. Four cosmids were found to cross the breakpoint. A new gene fusion generated by the t(17;22) in 11 DP and one GCF at the genomic and RNA levels, using Southern blotting, sequencing and RT-PCR was characterized.

34C

EXPRESSION OF T (THOMSEN-FRIEDENREICH) AND Tn ANTIGENS IN NORMAL AND NEOPLASTIC SKIN.

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The Thomsen-Friedenreich (T) and its precursor, the Tn antigen, are core disaccharides of complex carbohydrate antigens, whose expression seems to be correlated with prognosis of some human cancers. We have studied the immunohistochemical expression of these antigens in normal and neoplastic skin in order to evaluate their usefulness in the diagnosis of cutaneous tumors. Sections of normal skin, of inflammatory dermatoses and of 219 various cutaneous tumors were immunolabelled with the monoclonal antibodies HB-T1 and HB-Tn1. In normal skin the T (and at a lesser degree the Tn) antigen were regularly expressed in mature sebocytes, and rarely within the eccrine-gland excretory duct. Mesenchymal tumors (of lympho-histiocytic, pigmentary, nervous, vascular and muscular origin) were nearly always negative. T and Tn were detected in all sebaceous and in some sweat-gland tumors. Basal cell carcinomas were generally negative for both antigens; rare cases of squamous cell carcinoma expressed T but not (or very weakly) the Tn antigen. Metastatic adenocarcinomas (including Paget's disease) were Tn+/T± or -. These results show that the T antigen is a sensitive marker of sebocytes, useful for studying sebaceous differentiation. On the other hand, the predominant expression of the Tn (as compared with the T) antigen in metastatic tumors highlights the low degree of differentiation of tumor cells -that express the precursor (Tn) but not the complete (T) antigen-, and appears to be a useful adjunct for the differentiation between primary and metastatic skin carcinomas.

35C

SPECIFICITY AND CLASS I RESTRICTION OF CUTANEOUS LYMPHOMA INFILTRATING T-CELL CLONES.

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The aim of our study was to characterize the anti-tumor immunologic responses in cutaneous T-cell lymphomas. For several patients, we showed in the cutaneous infiltrate a proliferative and cytotoxic activity specific for the tumor cells of the patient. We report two tumor infiltrating clones, TC5 and TC7. These clones were CD4+CD8+ and CD4+CD8-, and had a tumor cell-specific proliferative and cytotoxic activity. Tumor cell lines have been cultured with IL-2 and IL-7, both from the skin and from the peripheral blood of this patient. The skin tumor cell line had a CD4+CD8- phenotype, and the blood tumor cell line had a CD8+CD4- phenotype. Both these tumor cell lines expressed MHC class I antigens but did not express MHC class II antigens. They were specifically lysed by TC5 and TC7. The cytotoxic and proliferative activity of TC5 and TC7 was blocked by the monomorphic anti-class I monoclonal antibody W6/32 but not by an anti-CD4 monoclonal antibody. TCR gamma rearrangement analysis showed the same clonospecific band for the cutaneous tumor cells and the skin- and blood-derived tumor cell lines, whereas TC5 and TC7 had a different profile. The study of the expression of TCR Vß genes showed that TC5 and TC7 expressed a unique transcript, corresponding respectively to Vß5/Jß2.3 and Vß17/Jß2.7. The tumor cells and skin- and blood-derived tumor cell lines expressed three transcripts Vß7/Jß2.3, Vß13/Jß2.5, and Vß22/Jß2.5. The analysis of the representation of the different Vß gene products showed that TC5 and TC7 were present in the cutaneous tumor before and after culture, but were not detected in the blood. These results demonstrate for the first time the presence within the cutaneous lymphoma T-cell infiltrate of CD4+ or CD4+CD8+ class I-restricted T cell clones. The role of these clones in the tumor progression remains to be determined.

37C

GENE REARRANGEMENT STUDY OF LYMPHOCYTIC DERMATOSIS BY PCR-CG clamp-DGGE. JP Buono, M D'Incan, C Picard, YJ Bignon, P Souteyrand, P Déchelotte. Laboratoire d'Oncologie Moléculaire INSERM CRI 9402 Centre Jean Perrin, Services d'Anatomie Pathologique et de Dermatologie Hôtel-Dieu Clermont-Ferrand, France.

Our aim was to study the clonality and the lymphocyte repertoire in cutaneous lymphomas and pseudolymphomas.

Method : TCR γ chain gene rearrangements were studied by PCR-CG clamp-DGGE using the multiplex procedure (to detect a clonal dominance) and PCR monoplex (lymphocyte repertoire study). Were included 43 skin and node biopsies from : 16 mycosis fungoides (MF) and Sézary syndrome (SS,), 13 pleomorphic lymphomas (PL), 6 lymphomatoid papulosis (LyP), 5 non cutaneous T-cell lymphomas and 3 pseudolymphomas. 12 biopsies were obtained from : 1 Hodgkin's disease and 11 benign lymphocytic dermatosis.

Results : A dominant clone was detected in 69% of MF and SS (11/11 infiltrated lesions, 0/5 lesions of recent onset), 91% of PL, 17% of Lyp, 80% of non cutaneous T-cell lymphomas and 0% of all other cases. Two dominant clones were detected in 92% of PL, 25% of MF and SS and 0% of LyP. The monoplex procedure detected 2 to 4 identical clones in LyP and PL lesions from the same patient and 3 to 5 identical clones in two different LyP lesions in another patient.

Comments : This study, 1- confirm that, even using PCR, a dominant clone is not detected in cutaneous lymphoma at the early stage, 2- suggests that the genotypic pattern of PL is particular and, 3- suggests the existence of a reactive non tumorous lymphocyte population in different lesions from the same patient whatever the histological type is. These tumor infiltrating lymphocytes clone could belong to a clone specific cellular immune response. Work is now in progress to characterize these lymphocytes.

38C

TAT PROTEIN REPRESSES METALLOENZYMES INVOLVED IN PEROXIDE METABOLISM AND INCREASED UV-A CYTOTOXICITY IN HELA CELLS. Richard Marie-Jeanne., P Guiraud, Favier Alain, Beani JC. Grepo (Research group on oxidative diseases). Faculté de Médecine-Pharmacie. Grenoble, France.

Several human immunodeficiency virus (HIV) gene products are important in the regulation of viral gene expression. Of these, the Tat (trans-acting transcriptional activator) and Rev proteins are essential for virus replication. On the other hand HIV infection is associated with oxidative stress. We explored the relationship between Tat expression, metalloenzyme involved in peroxides metabolism and UV-A irradiation.

Cells are submitted to UVA radiation, known to generate intracellularly hydrogen peroxide. We demonstrated that cytotoxicity of UV-A is increased in Hela-tat (lethal dose 50: 66 ± 3 J/cm2) compared to wild cells (lethal dose 50: 89 ± 2.5 J/cm2). The susceptibilty of Hela-tat to UVA is not linked to metal contents but seems to be due to a deep decrease in metalloenzyme involved in peroxide metabolism

We found that Hela cells which produce the HIV regulatory protein Tat (Hela-tat) have depressed levels of Glutathione peroxidase (GSH-Px) (31.5 ± 0.7 vs 12.3 ± 2.5 U/g prot wild compared Hela-tat cells) whereas intracellular selenium (Se) level was not affected. Catalase activity was also significantly affected (6.97 ± 0.37 vs 5.36 ± 0.58 U/mg prot wild compared Hela-tat cells) but less than GSH-Px. These deficiencies in antioxidant enzymes may contribute to the establishment and maintenance of oxidative stress when cells are exposed to pathological states involving free radicals production.

These data suggest that HIV could take control of the cell's antioxidant status and UVA could induce conditions necessary for successful viral replication.

39C

UVA MODULATE CELL ADHESION AND INTEGRIN EXPRESSION IN HUMAN DERMAL FIBROBLASTS. A.Tupet, C. Lebreton, L. Dubertret, B. Coulomb. Inserm U 312, Laboratoire de Dermatologie, Hôpital Saint-Louis, 75475 Paris Cedex 10 - France.

The interactions of cells with the extracellular matrix modify fibroblasts behavior. Particularly, they lead UVA radiations to have more pronounced cytotoxic effects *(Coulomb, 1996)*. The aim of this work was thus to study the effects of UVA radiations on fibroblasts adhesion and on the expression of given integrins subunits involved in collagen (α1, α2) or fibronectin (α5) binding. Fibroblasts, cultured for 3 or 4 days in monolayers, were irradiated with UVA (2, 10 and 20J/cm^2) and seeded onto collagen or fibronectin coated supports for 1 hour. Adhesion was then evaluated by counting adhering cells and integrin subunits expression was evaluated by immunolabelling.

-Fibroblasts adhesion is stimulated by 2J/cm^2 UVA radiation while higher doses (10 and 20 J/cm^2) inhibit this adhesion. PKC is involved in the mechanism leading to a stimulation of cell adhesion since GF109203X (a specific inhibitor of PKC in fibroblasts *(Le Panse, 1994)*) inhibits this effect, while PKC does not seem to be involved in the inhibition process.

-The integrins α1 and α2 subunits expression is usually stimulated by a 2J/cm^2 UVA radiation but α1 expression is inhibited if more than 20% of non-irradiated cells express α1. On the opposite, the effects of the UVA on α2 expression do not depend on the control expression. Higher doses of UVA always decrease the expression of α1, Concerning α2 expression, these higher doses (10 and 20J/cm^2) stimulate α2 when 2J/cm^2 inhibits α2, but they decrease α2 when 2J/cm^2 stimulates α2. Finally, α5 is generaly weakly expressed in controls, increased by 2J/cm^2 UVA radiation and then decreased for higher doses.

In conclusion, this work shows that the UVA radiation modify cell-matrix interactions by modulating adhesion properties and integrin expression. By studying signal transduction involved in these modulations, it will be possible to better understand the mechanisms by which the extracellular matrix modify fibroblasts behavior.

40C

AN EX VIVO STUDY OF CONGENITAL PIGMENTED NEVI ON RECONSTRUCTED EPIDERMIS. Bessou S[1], Morichon F[1], Surlève-Bazeille J.E[2], Bioulac-Sage P[3], Pain C[1], Taieb A[1,4]. [1]Laboratoire de Dermatologie, Université Victor Ségalen, Bordeaux II - [2]Département de Biologie Cellulaire et de Microscopie Electronique Université Bordeaux I - [3]Laboratoire d'Anatomie Pathologie - [4]Unité de Dermatologie Pédiatrique, Hôpital Pellegrin Enfants, Bordeaux, France.

Congenital pigmented nevi are present in around 1% of neonates. The biological behaviour of nevus cells as compared to normal melanocytes and melanoma cells remains debated. To study pigment cells from congenital pigmented nevi, we made autologous or heterologous reconstructions with normal keratinocytes on a dead deepidermized fermis. Nevus cells originated from the dermal epidermal junction or from the dermis. 19 series of experiments were performed with compound congenital pigmented nevi according to a technique published earlier (Pigm Cell Res 1995;8:241-9). When perilesional normal epidermis was not available reconstructs were made with keratinocytes of healthy donors, after culture and elimination of normal melanocytes. Nevus cells were used as fresh suspensions or after culture. Dermal nevus cells were obtained after enzyme treatment and nylon gauze filtration to remove hair follicles. Reconstructed epidermis were grown 15 days at the air-liquid interface with or without UVB exposure. A macroscopic, histologic and ultrastructural evaluation was performed. Only with non cultured cells and with those briefly cultured typical nesting of nevus cells was noted at the dermal epidermal junction or in the superficial dermis associated with macroscopically detectable smal pigmented macules. UVB exposure produced and upward migration migration of dermal nevus cells in the suprabasal layers of the reconstructed epidermis. These characters were lost after one week of culture of nevus cells prior to epidermal reconstruction.

Our data strongly suggest that the lost of environnemental factors in culture induces a reversal of the phenotype of nevus cells which is close to that of normal melanocytes. The alternative hypothesis, which seems less tenable, is that normal melanocytes exist even in the dermis and overgrow nevus cells in long-term cultures.

41C

p53 AND *MDM-2* GENE EXPRESSION IN HUMAN SKIN. J-E. Dazard[1,2], D. Augias[1], H. Neel[2], J. Piette[2], J-J. Guilhou[1], N. Basset-Séguin[1]. Laboratoire de Dermatologie Moléculaire[1], Institut de Génétique Moléculaire[2], Montpellier, France.

Recently, we have shown that the *MDM-2* gene is highly expressed in differentiated Normal Human Skin (NHS) and probably independently of *p53*. Here, we used several methods to detect p53 and MDM-2 proteins in order to define expression levels of both genes in NHS. Moreover, to better understand the relationship between *MDM-2* expression and keratinocyte differentiation, we have followed the expression of both genes in Psoriatic Skin (PS), a benign hyperproliferative pathology (n=13). The p53 and MDM-2 protein analysis was performed by immunohistochemistry (IHC) and Western Blot experiments (WB) by the use of a panel of monoclonal and polyclonal antibodies in NHS, in PS and *in vitro* in Reconstructed Skin (RS) from Normal Human Keratinocyte (NHK) and human tumor cell lines containing a mutated (HaCaT, A431) or degraded (KN20) p53 protein. Results were compared to that obtained from control cell lines : MCF-7 for p53 and 3T3DM for MDM-2. Our results have shown for p53 i) that the protein is not detectable by IHC in NHS, whereas its expression is clearly observed in RS from NHK, HaCaT, and A431 cells, ii) a significant amount of protein is detected by WB in all tissues studied suggesting that the epitopes are inaccessible for *in situ* detection or that the expression levels are too low. In contrast, we found a good correlation between the detection of MDM-2 in IHC and WB experiments in all tissues. Finally, p53 protein is undetectable by IHC in PS and the expression of MDM-2 protein is highly diminished in all proliferative layers. This study shows that significant yet different levels of p53 and MDM-2 proteins are found in NHS. Moreover, diminished expression of MDM-2 protein in psoriatic skin further supports its potential role in epidermal differentiation.

42C

EXPRESSION OF FOS/JUN FAMILY MEMBERS IN KERATINOCYTE CULTURE MODELS IN VITRO. N.Basset-Séguin, A.Tesniere, I.Tournillac, J.P.Molès J.J.Guilhou. Laboratoire de Dermatologie Moléculaire. I.U.R.C., Montpellier, France.

We have previously shown the importance of the c-fos proto-oncogene in keratinocyte differentiation. Fos and jun proto-oncogene belonging to a family of genes, we have studied in this work by immunohistochemistry using polyclonal specific antibodies against c-Fos, Fra 1, Fra 2, Fos B, c-Jun, Jun B and Jun D (generous gift from K.Pfarr, Institut Pasteur) the presence of these proteins in *in vitro* reconstructed tissues with normal human keratinocyte RE-KHN (differentiating normally) and tumoral keratinocyte RE-KHT (A431 cells which do not differentiate).Our results have confirmed that c-Fos is present in basal and suprabasal keratinocyte of RE-KHN. In this tissue Fra 1 and Jun D are detected throughout epidermal layers, as well as Fra 2 with increased intensity in the granular layer for this latter.Fos B and Jun B have a disperse pattern and c-jun is not detected. Conversly, in RE-KHT, c-Fos, c-Jun, Fra 1, Fra 2 are negative, jun D is always positive and Fos B and Jun B seem stimulated. Kinetic expression studies after serum stimulation of NHK show a rapid and sustained (<4h) expression of c-fos, Fra 1, Fra 2 and Jun B. Fos B staining is weak, c-jun is undetectable and jun D appears constitutively expressed.These results show that these gene are differentially expressed in reconstructed epidermis and that c-Fos/c-Jun heterodimers are not the major form. Additionnaly they bring further evidence of the role of c-Fos , Fra 2 and potentially Fra 1 in keratinocyte differentiation and show that jun D expression could be essential in keratinocyte regardless the state of the cell

*Abstract no. 36C is unpublished.

43C

INSULIN STIMULATION OF KERATINOCYTE HAPTOTACTIC MIGRATION INVOLVES ACTIVATION OF NFκB TRANSCRIPTION FACTOR. P. Verrando[1,2], B. Kahn-Perlès[1], A.M. Benolie[1]. 1. INSERM U387, 2 Laboratoire d'Investigation des Maladies de la Peau, 3 INSERM U119. Hôpital Ste Marguerite, 13274 MARSEILLE - FRANCE.

Insulin-mediated keratinocyte motility as well as the role of NFκB transcription factor in this process were examined. Insulin caused a dose-dependent stimulation of keratinocyte migration that maximally reached 2-fold at $2x10^{-7}$ M hormone. This phenomenon was independent of the nature of the extracellular matrix component on which the cell migrated, indicating that a specific integrin-ligand complex is not required.A 10^{-7} M insulin treatment of keratinocytes resulted in activation of a major κB DNA binding complex within 15 to 30 minutes, which was identified as the p65/p50 NFκB heterodimer by electrophoretic mobility shift assays. The activation induced nuclear translocation of cytosolic pools of NFκB factor. The use of two inhibitors of IκB(α) degradation which act by different processes (modification of cell redox status and inhibition of proteasome activity) reversed the insulin-stimulated keratinocyte haptotactic migration. The compounds inhibited the insulin-induced nuclear translocation of NFκB as detected by confocal laser microscopy. Taken together these experiments demonstrate for the first time that insulin is able to stimulate haptotactic migration of epidermal keratinocyte through activation of NFκB transcription factor, and provide a first clue to the mechanism of insulin-induced haptotactic migration.

44C

EXPRESSION CLONING OF THE HUMAN CORNEODESMOSIN. M. Guerrin, M. Simon, C. Vincent, and G. Serre. Department of Biology and Pathology of the Cell, INSERM CJF 9602-IFR 30, Toulouse-Purpan School of Medicine, Toulouse, France.

Corneodesmosin is a late differentiation protein of cornified squamous epithelia, defined by three mouse monoclonal antibodies (Mab), G36-17, F28-27, and B17-21, produced after immunisation with human stratum corneum. Its location in the corneocyte-specific reorganised desmosomes -corneodesmosomes- suggests a role for this protein in stratum corneum cohesion. An expression library was synthesized by oligo(dT) priming of poly(A+) RNA from human epidermis (2μg), and unidirectional cloning in lambda ZAP Express vector (Stratagene). Immunoscreening was performed without library amplification, using a cocktail of the three Mabs. Six independent clones were purified, tested with each Mab, analysed by restriction mapping, and sequenced by one run at both ends of the inserts.

Data analysis revealed that the six clones were overlapping, and established a preliminary map of the three epitopes defined by the Mabs, on the corneodesmosin sequence. BLAST data-base search indicated that corneodesmosin is identical to the product of the S gene, identified by CpG island analysis of the HLA class I region. However, complete sequencing of the clones revealed minor differences with the published S sequence, and predicted a slightly larger protein product (514 instead of 486 amino acids). Genomic analysis are being performed to elucidate these differences. Immunoblot analysis of Cos-7 cells, transiently transfected with the corneodesmosin cDNA, identified a unique protein with an apparent molecular weight of 60 kDa, slightly higher than the 56-52 kDa form extracted from epidermis.

Surprisingly, cloning also revealed that corneodesmosin is a highly serine- and glycine-rich protein (near 30% and 15%, respectively). Structural domains composed of extended glycine loops have been described in other epidermal proteins (cytokeratins and loricrin) and are supposed to be of highly flexible nature. These motives could account, at least in part, for the mechanical properties of the corneodesmosomes.

45C

PURIFICATION OF HUMAN EPIDERMAL CORNEODESMOSIN, A BASIC PROTEIN OF CORNEODESMOSOMES. M. Montézin, M. Simon, M. Guerrin, G. Serre. Department of Biology and Pathology of the Cell, INSERM CJF 9602-IFR 30, Purpan School of Medicine, Toulouse, France.

Recent studies have shown the major role of corneodesmosomes in corneocyte cohesion. We have identified a new protein specific of these structures: the corneodesmosin. It is synthesized as a precursor of 52-56 kD in the granular keratinocytes, then incorporated in corneodesmosomes and proteolyzed during the maturation of the stratum corneum. The last step of the proteolysis in the uppermost corneocytes seems to be a key event in desquamation.

The purification of this basic protein (pI > 8.5) from normal human epidermis was initiated by extraction of the precursor in a hypotonic buffer. The extract was injected on an anion exchange column at pH 7.5. Flowing through proteins (almost 5 % of the extracted proteins) were then injected on an affinity column which was coupled with a specific monoclonal antibody (G36-19 or F28-27). Immunoadsorbed proteins were eluted at acidic pH. Corneodesmosin was found to be highly preponderant in the eluted fractions. The purification yield was greater than 90 %. A preparative bidimensional electrophoresis was performed to achieve purification to homogeneity. Corneodesmosin was estimated to be 1/1,000 of the extractable protein mass. The purified protein was very unstable both at room temperature and at 4 °C. It could be stabilized by addition of SDS or protease inhibitors.

Sequencing of the amino-terminal end, and of internal peptides produced by proteolysis of the purified protein is currently in progress. The results should confirm the sequence obtained by cloning and, in particular, that the 52-56 kD protein is produced after cleavage of a signal peptide.

47P

CYCLOSPORIN A INHIBITS INDUCIBLE NITRIC-OXIDE SYNTHASE (iNOS) INDUCTION AND TNF-α RELEASE IN HUMAN KERATINOCYTES.

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Cyclosporin A is used in the treatment of systemic and cutaneous autoimmune diseases, such as psoriasis, severe erosive lichen planus or acute GVHD, both in topical or systemic administration. Generation of NO is thought to be an important event during inflammatory response of various cell types including human keratinocytes. It has been suggested that iNOS was expressed in psoriatic keratinocytes, and we have previously demonstrated that retinoids exerted their anti-inflammatory effect through inhibition of iNOS mRNA expression in LPS/IFN-γ activated keratinocytes. We here investigated the effects of CyA on iNOS expression by LPS (1 μM)/IFN-γ (1000U/ml) stimulated human keratinocytes. The release of nitrites in culture supernatants was inhibited in a dose dependent manner (CyA: 10^{-5}-10^{-1} M, inhibition: 12-80%) after 48 and 72 h. The synthesis of TNF-α was also inhibited by 75% (140 to 30 pg/ml) after cell preincubation with CyA. The enzymatic conversion of ^{14}C-L-Arginine to ^{14}C-L-Citrulline was strongly down-modulated (110 pmol/mn/mg to 35 pmol/mn/mg), and this inhibition was not due to a direct effect of CyA on the enzyme activity. Actually, RT-PCR experiments confirmed that the iNOS mRNA was down modulated after an incubation of 18h with CyA. These results suggest that the clinical anti-inflammatory effects of CyA might in part be explained by the inhibition of iNOS gene transcription in inflammatory keratinocytes and subsequent TNF-α synthesis.

48P

EFFECT OF DERMAL INTOXICATION BY BCES: AN IN VITRO STUDY. A. Réano[1], E. Gentilhomme[2], J. Bergier[2], D. Pradel[2], Y. Neveux[3], D. Schmitt[1]. 1: INSERM U.346, Hôp. Ed. Herriot, Lyon, 2: C.R.S.S.A., La Tronche Grenoble, 3: H.I.A. Val de Grace, Paris, France.

In human, direct skin intoxication by yperite, Bis(betachloroethyl)sulfide (BCES), induces lesions similar to thermal burns, characterized by slowness of cicatrisation. We have developped an in vitro model of skin equivalent to investigate some of the mechanisms involved in this process. Human dermal fibroblasts were seeded into type I collagen gels and the lattices were cultured without tension. They were exposed on the seventh day to concentrations of BCES ranging from 5.10^{-6} to 5.10^{-3}M in DMEM, in parallel to negative controls (DMEM alone and solvant 0.5% ethanol in DMEM) and supplementary lattices irradiated by gamma ray with a single dose of 50 Gy which serve as positive controls. Direct intoxication of dermal equivalent lead to a dose and time dependant cytotoxicity. A decrease of macroscopic retraction of collagen lattices was observed, parallel to the toxic concentration, with, at the histological level, absence of collagen fibers reorganization. Fibroblast biosynthesis of fibronectin was also inhibited upon intoxication by BCES, appreciated at the immunobiochemical and immunohistochemical level as well as the expression of tenascin. These dermal alterations were correlated with secondary troubles of epithelial maturation of non intoxicated normal human keratinocytes. Cellular adhesion was perturbed, as visualized by a delay in the expression and the reorganization of basement membrane components: laminin, collagen IV and fibronectin. Epidermal maturation was also affected, as shown by the absence of terminal differentiation markers. We thus demonstrated, in this in vitro model, that direct dermal alterations secondarly induce maturation disturbance of untreated keratinocytes. These data illustrate the key role of dermal-epidermal interactions in the normal skin reconstruction. Clinically, this can explain slowness of cicatrisation observed after human skin injury by BCES.

49P

MODULATION OF IgE-DEPENDENT HYPERSENSITIVITY REACTION BY MIZOLASTINE: INHIBITION OF ANTIGEN-INDUCED SOLUBLE ICAM-1 RELEASE IN VIVO. L. Michel*, L. Dubertret*, F. Jean-Louis*, M. Murrieta-Aguttes+, D. Tudor+. * INSERM U312, Hôpital Saint-Louis, Paris; +Synthélabo Recherche, Bagneux, France.

Mizolastine (M) is a new non-sedative H1 antagonist proven to be effective and safe in the treatment of allergic rhinitis and urticaria. The purpose of the study was to quantitatively analyze the inhibitory effects of mizolastine on the development of IgE-dependent hypersensitivity reaction induced by antigenic challenge in vivo. Twelve pollen-sensitive patients (23±6 yrs) were included in a double-blind cross-over study versus placebo (P). Patients orally received 10 mg M or P, once daily, for 5 days, during the first period and vice versa after a 3 week wash-out period for the second period. On day 4, by using a non invasive skin chamber technique, the relase of mediators such as histamine, tryptase, soluble ICAM-1 (sICAM-1), vascular permeability alterations or inflammatory cell recruitment were investigated at skin sites challenged with grass pollen or exogenous histamine during 24 hours. Statistical analysis of results showed no carry-over effect. Results after 2 hours and during the first 8 hours of challenge show that although M compared to P did not alter histamine or tryptase release, it significantly inhibited the early and late sICAM-1 release after sustained challenge with grass pollen (after 2h: $p \leq 0.03$) or histamine (after 2 and 8h: $p \leq 0.05$). In addition, M significantly inhibited protein extravasation after stimulation with either pollen ($p \leq 0.04$ at 2h, $p \leq 0.003$ at 8h) or histamine (after 2h: $p \leq 0.003$, after 8 h: $p \leq 0.009$). M also significantly decreased the monocyte recruitment observed on the superficial dermis 24h after antigenic challenge ($p \leq 0.035$). In conclusion, although M did not alter cutaneous mast cell degranulation *in vivo*, it reduced the early and late allergic response such as sICAM-1 release or protein extravasation as well as inflammatory cell recruitment during IgE-mediated reactions in the skin.

*Abstract no. 46C is unpublished.

50P

Effect of mizolastine vs placebo in Acquired Cold Induced Urticaria (ACIU) : the cold stimulation time test (CSTT)

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L. Michel

Cold urticaria (CU) is a rare form of physical urticaria. The ice-cube test is used for the diagnostic of most CU and the CSTT is defined as the minimum time of cold contact stimulation required to induce an immediate coalescent wheal (CW). 28 patients (35 ± 17 yrs) suffering from ACIU for 6 ± 8 yrs were included in a double-blind cross-over placebo (P)-controlled study to evaluate the effect of M, a new H1 antagonist, effective in allergic rhinitis and urticaria. Patients received M10mg or P o.d. in the 1st period for 7 days and *viceversa* in the 2nd period. On day 0,7,21 and 28 an ice-cube was applied in the forearm for time interval up to 15 min., until a CW was induced. The diameter of wheal was measured 5,10 and 20 min., after removed the ice-cube. When a positive test was elicite it was repeated at 3,6 and 10 min.intervals in order to stablish the CSTT. The sev. 'v of prurit was evaluated using a visual analog scale [(VAS) 0-10 cm]. Statistical analysis showed no carry-over and no sequence effects. The intent-to-treat analysis showed that M compared to P :

- delayed the cold-induced whealing reaction (p=0.006 vs P) with a CSTT>15 min. for 36% of M responder patients vs 14% with P(p<0.05).
- decreased the CW at 3 and 10 min. (p<0.01 and p<0.009).
- decreased at least 50% the total score of wheal response (sum of diameter measured at 3,6 and 10 min.) in 67% of M patients vs 30% with P (p<0.001).
- decreased the cold induced pruritus (p<0.005 vs P).

In conclusion, in comparison to P, mizolastine decreases and delays the cold induced coalescent wheal measured by a ice-cube test in patients with ACIU.

51P

FOLLOW OF THE THICKNESS EVOLUTION OF THE ADIPOSE TISSUE OF THE EXTERNAL FACE OF THIGH IN WOMAN BY ULTRASOUND IMAGING

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The aim of this study was to verify, on a reference group of 30 women, the existence and the extent of thickness variations of the adipose tissue localised on the external thigh face. Followed parameters during 3 months : 1) the thickness of the adipose tissue of the thigh external face by ultrasound imaging (14 Mhz) ; 2) the perimeter of thighs ; 3) the weight. Selected women, were menstruated, had a stable contraception and weight (20 <BMI< 25). Examinations have been realised every 15 days. Echographic results demonstrated that 60% women exhibit significant variations of the thickness of the subcutaneous tissue (2 mm ; 6%), related to the menstrual cycle. These variations appeared precisely 4 days before or during the period of menstruation. Evolution of the perimeter and the weight confirmed these results. This study has allowed:

1) to confirm the interest of the ultrasound imaging for a such approach ;
2) to demonstrate the existence of a relationship between the menstrual cycle and the thickness of the adipose panicle ;
3) to confirm the necessity to take into account these fluctuations in test efficiency test of slimming products.

Because of the phase difference of the cycle for each woman in a same group (min15), this periodic thickness variation does not appear for average values. This verification allows therefore the realization of efficiency tests, "group to group" (treated / ref. or treated / placebo) or by the comparison of the treated and the reference or placebo leg of a same woman. In this last case individual values are equally independent of these cyclic thickness variations.

52P

EFFECT OF AN EXTRACT OF CYANOPHYCEAE ON THE CUTANEOUS CELLULAR STIMULATION.

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The cellular stimulation activity of an extract of Cyanophyceae is evaluated by several assays using monolayer cutaneous cellular cultures, and the building of an epidermal sheet in vitro.

The cellular growth of several kinds of epithelial and fibroblastic cells is measured by a spectrophotometric method, using the test of transformation of MTT, after treatment at several concentrations of the extract of Cyanophyceae, against EGF as reference product. A test of metabolization of XTT by a determined population of fibroblast in culture, completed by an oxygraphy method with measure of oxygen used by cells, using a Clark electrode, allows to assess the effect of this extract on the cellular metabolism.

The epidermic sheet is built from a human monolayer keratinocyte culture on polycarbonate support, at increased concentrations of extract and against EGF as reference. After 14 days of culture, including 6 in submerged conditions, sections are made for histological treatment (hematoxyline-eosine coloration) and for immunofluorescence (Ki67, propidium iodide).

The present extract of Cyanophycae has a dependent-dose effect on the main tested methods, its average of activity is about 10 µg/ml.

The association of theses various methods obviously allows to show that the stimulating activity of this extract implies a restart of cellular metabolism.

The active parts of this extract are now under identification process.

53P

SKIN VASCULARIZATION STUDY BY THE MEAN OF ULTRASONIC TECHNIQUES.

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The objective of this study is to adapt the ultrasonic techniques to the specificities of the skin microcirculation, that is flow velocities in the mm/s range and vessel size in the µm range.

Measuring blood flow at well defined sites in human skin is still an unsolved problem. Conventional ultrasonic Doppler techniques are not suitable for this type of measurement because of long acquisition time and low spatial resolution compared with the tiny vascularization structure of the skin, and then cannot be easily used in clinical situations. In other respects, Laser Doppler do not provide any accuracy for the location of the measured volume. A dedicated technology based on a 20 MHz echographic imaging system has been developed. In order to reach the desired performances for future in-vivo work it will be shown how the experimental data, i.e. the radiofrequency backscattered signal, has to be analysed. Special care has to be paid to remove the artefacts caused by muscle tremulousness and respiratory movements.

In-vitro results show that velocity measurements as low as 0.1 mm/s are attainable with 80 µm axial resolution and 200 µm lateral resolution.

When transposed in-vivo, this technique will allow a more precise exploration of circulatory troubles in cutaneous pathologies.

54P

PHONOPHORESIS OF SALICYLIC ACID AND HYDROCORTISONE UNDER HYDROCOLLOID DRESSING .

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Hydrocolloid dressings are used alone or associated with corticosteroids in the treatment of psoriasis. The aim of this study was 1) to study percutaneous diffusion of salicylic acid and hydrocortisone when administred under a hydrocolloid dressing, and 2) to investigate the additional effect of ultrasound on percutaneous delivery.

Sonication was carried out at 1.5 W/cm2, 1.1 MHz for 20 minutes. We used modified Franz diffusion cells adapted for sonication. During sonication the temperature in the donor compartment was continuously monitored and maintained at 31°C by the cooling coil. Diffusion of tritiated hydrocortisone and 14-C salicylic acid across whole human skin was determined by liquid scintillation, and the steady-state flux (pg/cm2/h ±SEM) was determined.

Mean steady-state fluxes are expressed in the Table.

	control	dressing	dressing+ultrasound
Hydrocortisone	3.0±0.6	3.2±0.5	3.6±0.5
Salicylic acid	40.5±4.5	43.2±4.2	46,3±3.7

No increase in steady-state diffusion rates was observed in an ex vivo assay with the 2 drugs. Clinical improvement observed in open in vivo studies with hydrocolloid dressings associated with dermocorticoid may be due to factors other than increase in percutaneous diffusion.

55P

ECHOGRAPHIC DEMONSTRATION OF FACIAL OEDEMA IN ADULTS AFTER 24 HOURS OF ANTI-ORTHOSTATIC BEDREST AT -10°.

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We were interested in investigating the appearance of facial oedema in subjects undergoing anti-orthostatic bedrest at an angle of -10°.

Four subjects were measured on the forehead before, and after 1, 10 & 24 hours in this supine position. During this time, interstitial fluid migration and facial oedema were assessed using a high resolution B-scan ultrasound (Microscan - 20 MHz), and a device for measuring the skin's mechanical properties.

The results obtained showed a progressive increase in dermal thickness, suppleness and tension, and a reduction in elasticity during the study period.

This preliminary study has demonstrated the feasability of the method in measuring fluid displacement and retention in the skin. Furthermore, it highlights the influence of fluids on the mechanical behaviour of the skin.

We hope to be able to use these techniques for studying the redistribution of liquid masses during periods spent in space.

56P

SEASONAL AND ANATOMICAL VARIATIONS IN THE SURFACE STATE OF THE STRATUM CORNEUM.

D.Black, A Del Pozo*, Y.Gall. IRPF, Centre Jean-Louis Alibert, Toulouse, France; (*) Department of Pharmacy, University of Barcelona, Spain.

Twenty-five women aged 18 to 34 with normal skin were used to investigate the influence of season on the surface state of the stratum corneum. Volunteers were included who used little or no body cosmetics, with facial cosmetics being allowed except during assessment periods.

During February, April, July and December 1995 in Toulouse, three sites were studied; the crow's foot area of the face (CFA), the mid volar forearm, and the calf.

On each site, the following measurements were made : skin surface capacitance (Corneometer CM 420), transepidermal water loss (Evaporimeter EP1), squame morphology (D-squame + image analysis), surface roughness (replicas + image analysis), and corneocyte number (epidermometer + counting).

Differences with season, mainly between July and the other months, were seen with calf and forearm sites, but not with the CFA. This was true for all parameters except surface roughness, which showed no seasonal variation. The results indicated that calf skin was driest and flakiest in winter, with forearm skin being similar but less pronounced. These two sites improved in summer, approaching that of the CFA which remained well hydrated with regular desquamation at all times.

Temperature, sunshine hours, and absolute humidity were greatest in July, which may explain the differences seen on calf and forearm sites. With regard to the CFA, the use of facial cosmetics may also be a factor.

57P

CORRELATION BETWEEN EPIDERMAL HYDRATION, TENSION AND SUPPLENESS OF THE SKIN.

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The aim of this study was to correlate the hydration, tension and suppleness of skin after water-in-oil emollient application, using *in vivo* assessment techniques.

The study was of randomised, open design, involving treated and untreated (control) volar forearm sites of 10 healthy volunteers. Measurements were made before treatment (0h), and after ¼, 1¼, 2¼, 3¾, & 5¾ hours of emollient application. These were made using an NMR imager-spectrometer (Biospec-Bruker) which measures the transverse relaxation time T2, a good indicator of the hydration state of the inner and outer epidermal layers; a high resolution B-scan ultrasound (Microscan 20), a cutometer and a corneometer (Courage & Khazaka).

The results show that "superficial epidermal hydration" and skin tension are changed after ¼h of product application. A maximal effect on skin suppleness and "deep epidermal hydration" is seen after 2¼h.

This study relates the hydration of the stratum corneum and the epidermis to the mechanical behaviour of the skin, and provides a better understanding of the time course and effects of emollients on skin.

58P

IN VIVO IMAGE ANALYSIS USED TO MEASURE SWEATING.

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The aim of this study was to quantify sweating activity, not only in terms of gross amounts produced such as that measured by gravimetric techniques for example, but also in terms of individual gland activity. We have thus developed an image analysis technique applicable to the assessment of sweat gland activity via the starch/iodine/sweat reaction. This technique has been used for measurements on the sole of the foot.

Using a videomicroscope fitted with a X50 objective, images of black sweat droplets on a yellow/brown background can be acquired.

We present here the image treatment procedure which enables the sweat droplets to be separated from the background, and subsequently quantified. Using this technique, two anti-perspirant products were evaluated, these being randomly allocated to each foot in a group of 17 subjects, and applied for 3 weeks.

Both products proved effective in reducing sweating, one moreso than the other in terms of the number, total surface area and size distribution of droplets.

59P

ASSESSMENT OF DRUG IRRITATION BY SLS- TANDEM APPLICATION : EXPERIENCE WITH RETINOIC ACID AND PENETRATION ENHANCERS.

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The combination of topical drug application and surfactant skin injury, with evaluation by bioengineering techniques, could be of great interest as a maximized assay to assess tolerance to topical products. One approach is to measure the ability of the drug to disrupt the skin barrier, i.e. to promote the response to a known irritant applied subsequently. Another is to evaluate the effects of the drug on a skin previously made more sensitive with an irritant.

We applied drug penetration enhancers (laurocapram, propylene glycol and SEPA) before skin exposure to 0.5% SLS. In a second study, 0.05% retinoic acid was applied after skin pre-treatment with 1% SLS. The variations of biophysical skin parameters (trans epidermal water loss, erythema, capacitance, blood flow) and visual scoring were investigated. Chemicals were applied for 24h under occlusion on the back of healthy volunteers. Controls were performed for every experiment.

Both RA and SLS irritant potential were confirmed. However, the variations of the investigated parameters were dissimilar, suggesting different pharmaco-toxicological mechanisms. In SLS pre-exposed skin, TEWL, erythema and scaling responses to RA were increased. On the contrary, the application of the various drug penetration enhancers did not induce a significant difference in SLS irritation, compared to their respective vehicles.

Overall, in our test the different chemicals ranked in the expected order according to the previous data available. However, the second approach seems to be more efficient. Furthermore, extensive SLS injury may mask the skin response to certain chemicals. These results suggest that the level of pre-irritation should be adjusted to the irritant potential of the chemicals tested.

60P

ESTIMATION OF THE ATTENUATION PARAMETER FROM ECHOGRAPHIC BACKSCATTERED SIGNALS IN HUMAN SKIN EX VIVO.

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Tissue characterization consists in quantifying an acoustic parameter for a differentiated diagnosis between healthy and pathological tissues. In this study, we estimate the attenuation parameter on healthy skin samples that were excised on three women abdomen.

Our transducer has a central frequency of 35 MHz, a lateral and axial resolution of 85 and 40 µm and is fixed on a 3 axis positioning system. Skin samples are immersed in a normal saline bath (NaCl 0.9%), and insonified. Several set of 256 backscattered A lines are acquired on each sample in reflexion mode. Diffraction is corrected. An appropriate signal processing method can allow us to have access to the mean attenuation parameter in the dermis (Table 1).

Table 1 : Mean attenuation measurements (dB/cm.MHz).

Age	Acquisitions sets	Mean attenuation
33 years old	7	2,7 +/- 0,5 dB/cm.MHz
42 years old	7	2,5 +/- 0,3 dB/cm.MHz
56 years old	8	2,4 +/- 0,4 dB/cm.MHz

Preliminary values in human skin are a little bit lower than that found in the literature concerning porcine skin (1). We notice a slight decrease of the attenuation parameter with age.

61P

MOSQUITO BITE OF AEDES AEGYPTI INDUCES A PRURITUS AND AN ERYTHEMA MORE IMPORTANT THAN ANOPHELES STEPHENSI'S BITE.

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The aim of this study was to select the more suitable mosquitoes species for evaluating the effect of antagonists on human cutaneous reaction after mosquito bites.

Six healthy adult volunteers (aged 20 to 24 years) who did not had previous history of allergy were exposed to 2-3 mosquito bites on both forearms. Each forearm was bitten either by Aedes aegypti or by Anopheles stephensi, 2 mosquito species from different biotope. Intensity of pruritus was assessed on a visual analogue scale and the erythema was evaluated by the technique of chromametry. All measurements were done during 90 min and at 24 hours after mosquito bite.

The results show that: the evolution curve of pruritus induced by A. aegypti increases strongly and longer than the one induced by A. stephensi, the characteristic chromametric parameters of the erythema vary more intensively in the case of A. aegypti.

As A. aegypti induces a pruritus and an erythema more important than A. stephensi, this kind of mosquito seems to be the more appropriated to the study of anti-inflammatory substance efficacy.

62P

SJÖGREN'S SYNDROME : DRY SKIN OR ASTEATOTIC SKIN?

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The aim of our study was to demonstrate the prevalence of dry skin in Sjögren's syndrome (SS) and wether it is determined by hypohidrosis or asteatosis.

Patients and Methods :

We included 11 women aged from 45 to 76 years with SS, 11 age-matched controls(+/- 5 years), and 12 patients with xerostomia due to drug (n=5), sarcoidosis (n=2) or idiopathic conditions (n=5). The appearance of the skin was evaluated semi-quantitatively (0 to +++) according to xerosis and desquamation. We measured epidermal hydration by electrical capacitance (Corneometer®), sebum excretion rate (SER) on the forehead (Sebumeter®) and cutaneous thickness by echography and skin extensibility (Cutometer®).

Results

Dry skin was found in 9 cases on 11 and in 10 cases the SER was low (< 90), whereas dry skin was found in only 2 xerostomia and 0 control ($p<0.01$). Epidermal hydration (79 vs 84), cutaneous thickness (0.99 vs 1.06 mm) and skin extensibility were not different in patients and controls. SER was statistically different ($p<0,05$) in SS (45.7± 33) and in controls (80,5 ± 55,7), but not in the xerostomia group.

Comments

Our study demonstrate that the prevalence of"dry skin" is high in SS. The absence of abnormalities in epidermal hydration and skin extensibility suggest normal cutaneous hydration in SS. On the other hand, the low SER could explain the clinical aspect of dry skin and asteatosis may be responsible for the "dry skin" and pruritus in SS.

63P

MEASUREMENT OF CUTANEOUS THICKNESS IN PATIENTS WITH SCLERODERMA USING 20 MHz ECHOTOMOGRAPHY.

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Scléroderma is a systemic disease characterized by an excess of collagen in the skin and other organs such as lung and oesophagus. A recent study has demonstrated that serum III procollagen (SPIIINP) (which is an aminopropeptide of type III collagen), level was increased in patients with scleroderma.

The purpose of the present study was to measure the cutaneous thickness in 40 patients using high resolution echotomography. The cutaneous thickness was obtained from the back of the right hand and the upper part of the thorax (mecchanical probe 20 MHz, axial and lateral resolution of 80µ, GIP Ultrasons, TOURS). The SPIIINP level was determined by radioimmuno assay (IRMA) The cutaneous thickness from the back of the hand was lower than from the upper part of the thorax in 37/40 patients. A trend to an increase in cutaneous thickness with the extension of scleroderma was noted. A significant correlation between cutaneous thickness and SPIIINP level was observed ($r=0.69$ $p<0.05$).These results indicate that the cutaneous thickness assessed by echotomography could be valuable to evaluate the cutaneous infiltration and could be taking into account as a new method of scleroderma reach.

64P

VARIABILITY OF FATTY TISSUE THICKNESS MEASUREMENT USING ULTRASONOGRAPHY

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The recent development of efficiency tests in cosmetology has entailed the improvement in imaging techniques to appreciate some cutaneous parameters such as dermous, hypodermous or fatty tissue thickness.

The purpose of the present study was to evaluate the variability in subcutaneous tissue ultrasonographic measurements using different ultrasound frequencies (10, 14 MHz), different fields of view (37, 50 and 73 mm), different numbers of measurements by acquisition (3 to 20) and to test the influence of sonographer practice.

The development of a precise beam contention system has allowed to obtain the best reliable measurements using ultrasound frequency of 10 MHz, a field of view of 50 mm, the need of 3 image acquisitions with 3 thickness measurements on each. This method ensures the lowest variability which was better than 1 mm (<3%).

This study shows that high frequency ultrasonography with a rigid protocol is a well-adapted method to evaluate the thickness of subcutaneous adipose tissue and its follow-up under specific treatment.

65P

EFFECTS OF WATER AND ORGANIC SOLVENTS ON HUMAN SKIN SURFACE
(*In vivo* study)

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In the laboratories as well as in the industry the usage of water, and organic solvents is daily. These liquides are often in contact with the skin surface.

In this work, we detected and evaluated the effects of these solvents on human skin surface. Qualitative and quantitative analyses of the skin surface structure were performed on skin replicas by using Scanning Electron Microscopy (SEM) and Confocal Optical Microscopy (COM).

There was a good correlation betwen the two results obtained by the two techniques. For example : Water decreased the depth of furrows and increased the spacing betwen them. The Ethanol attacked the cells membranes and decreased the furrows depths, giving slight swelling on the skin surface.

66P

ACTIVITIES OF DIFFERENT *AVENA RHEALBA* EXTRACTS ON INFLAMMATORY LIPIDS MEDIATORS AT THE CUTANEOUS LEVEL

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Eicosanoids (prostaglandins and leukotrienes) represent a large and heterogeneous family of oxygenated C20 fatty acid metabolites derived mostly from arachidonic acid (AA). They are biologically potent agents acting at/or near the site of their synthesis and are involved in inflammation, wound repair and cellular proliferation in various tissues including skin. Eicosanoid formation seems to be a common response of the skin to all kinds of skin irritants and injuries. Dysregulation of epidermal eicosanoid biosynthesis has been observed in inflammatory skin disorders.

The aim of this work was to evaluate the activity of different preparations of *Avena Rhealba* extracts (dry or moist process or NaOH extraction) on the inflammatory response of human keratinocytes in particular on the arachidonic acid cascade. In the present study, SVK14 human epidermal cell line was firstly exposed to *Avena Rhealba* extracts (0.01%, 0.05%, 0.1% concentrations in culture medium) for 1 hour, and then stimulated by the calcium ionophore A23187 for 5 hours. The inflammatory response was followed by the evaluation of 6KF1α prostaglandin, a major metabolite of keratinocyte arachidonic acid cascade produced from cyclooxygenase pathways, in the cell supernatants by enzymatic immunoassay (EIA).

Each *Avena Rhealba* extracts significantly inhibited the production of PG6KF1α and this activity was dose-dependent.

These results demonstrate that *Avena Rhealba* is able to modulate the cutaneous inflammatory reaction especially through its inhibitory activity on 6KF1α prostaglandin, stable metabolite of prostacyclin, with well-established vasoactive properties.

67P

RELATIONSHIP BETWEEN THE DAILY NUMBER OF NEWLY FORMED BULLAE AND THE PRESENCE OF CIRCULATING ANTI-BPAG2 ANTIBODIES IN PATIENTS WITH BULLOUS PEMPHIGOID.

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Introduction : The pathogenicity of anti-BPAG2 antibodies has been demonstrated in bullous pemphigoid (BP).

Design : To investigate the relationship between the daily number of newly formed bullae and the presence of circulating anti-BPAG2 antibodies in BP patients.

Patients and Methods : 68 BP patients with typical clinical and histologic features and DIF pattern of BP have been included. The precise number of daily formed bullae was estimated over a three days period before treatment. Sera were studied by immunoblotting using human epidermal extracts. The immunoblots were read independently by three investigators and compared to the number of bullae by the non-parametric statistical test of Mann & Whitney.

Results : Ten patients whose immunoblot interpretation was divergent from one investigator to another, were excluded. This was almost exclusively due to different interpretations of a weak to very weak band co-migrating with BPAG1. Among the 58 remaining patients, the mean number of newly formed bullae was 47.7 /d (median = 15 /d) for patients with circulating anti-BPAG2 antibodies (n = 27) and 10.0 /d (median = 3 /d) for those without anti-BPAG2 antibodies (n = 31). Interestingly, 11 of 12 patients with more than 40 bullae/day had circulating anti BPAG2 antibodies. No correlation was found between the number of bullae and the presence of anti-BPAG1 antibodies : BPAG1 (+) : 24.2 /d (n = 37); BPAG1 (-) : 33.5 /d (n = 21).

Comments : These results suggest relationship between the presence of cirulating anti-BPAG2 antibodies and the daily number of newly formed bullae in BP patients.

68P

IMMUNOBLOT ANALYSIS OF SERA FROM PEMPHIGUS PATIENTS WITH CANCER. P. Joly, D. Gilbert, E. Thomine, A. Delpech, Ph. Lauret, F. Tron. Groupe de Recherche en Immunopathologie (GRIMP) - ROUEN.

Introduction : Paranéoplastic pemphigus (PNP) is a newly described type of pemphigus associated with neoplasia.

Design : 1) To analyze the antigenic specificities identified by sera from patients with pemphigus associated with neoplasia; 2) to tempt to correlate the antigenic specifities recognized by the sera with the type of pemphigus and the type of neoplasia.

Patients et Methods : Sera from 29 patients with pemphigus associated with neoplasia were analyzed by immunoblotting, using bovine tongue and human epidermal extracts as the substrates.

Results :

Type of *pemphigus*	Type of neoplasia			Antigenic specificities recognized by sera (-kD).					
	LPD	Thym.	Carc.	250-210	200	190	160	130	Nég.
PNP (17)	14	2	1	17	4	7	0	4	0
PV (9)	0	2	7	0	0	3	1	6	3
PF (3)	0	2	1	0	0	0	1	0	2

Discussion : This study demonstrates that: 1) PNP is not the only type of pemphigus in patients with neoplasia; 2) there is an overlapping distribution of autoantibody specificities between the different types of pemphigus; 3) PNP is preferentially associated with lymphoproliferative disorders (LPD): 14/17, whereas PV are most often associated with carcinomas (carc): 7/9 and PF with thymomas (Thym): 2/3, although thymomas may be associated with all types of pemphigus.

69P

DETERMINATION OF SUSCEPTIBILITY FACTORS IN PEMPHIGUS AND BULLOUS PEMPHIGOID: GENETIC POLYMORPHISM OF IMMUNOGLOBULIN HEAVY AND LIGHT CHAIN GENES.

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Pemphigus and bullous pemphigoid form a group of autoimmune blistering skin disease characterized by the production of autoantibodies directed against desmosomal and hemidesmosomal antigens and the subsequent formation of blisters. To investigate whether the immunoglobulin heavy- (H) and light- (L) chain genes influence the occurence of these diseases, the immunoglobuline γ and κ locus polymorphism was studied in patients and healthy controls. Three groups of caucasian individuals were constituted: (i) 16 patients with pemphigus, (ii) 21 patients with bullous pemphigoid, and (iii) 35 healthy individuals ethnically matched to the 2 disease groups. Using the restriction enzymes BamH1, Sac1, EcoR1, and BstEII and specific probes for constant regions of the γ locus, we spanded several restriction fragments in the immunoglobulin heavy chain γ locus, defining the RFLP alleles for the immunoglobulin heavy-chain γ genes. In parallel, allotypic markers of κ chains were determining using a method combining specific amplification of the constant segment Cκ by PCR and subsequent restriction enzyme digestion. Allele frequencies of both H and L chains were compared between disease and control groups, allowing to determine susceptibility factors for the occurence of these diseases.

70P

A LONG TERM STUDY OF PREDICTIVE FACTORS FOR FAILURES IN 237 PATIENTS WITH ACNE TREATED BY ISOTRETINOIN

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To study the relationship between possible predictive factors such as the microcystic character of acne, endocrinological disorders, dosing schedule, and the occurrence of either early or late relapses, 237 patients (mostly females) were treated with daily dosages according to clinical and biological tolerance. Duration of the treatment ensured whenever possible an efficient total cumulative dose. Acne was graded initially, at the end of the treatment and one year thereafter. Failure was defined as the persistence or the occurrence of inflammatory lesions graded at 2 or more. Complete clearing at the end of treatment was observed in 218 patients. Correlations between clearing and age, sex or endocrinological disorders could not be established. Patients with microcystic acne cleared less. Failures have been recorded in 120 patients. Relapses were more frequent in patients with microcystic acne or endocrinological disorders as well as in those with high graded acnes on the face, on the back and on the chest. No relationship was found between total or daily dose and occurrence of failures. Definitive clearing was more frequent in patients who received a total cumulative dose between 75 and 150 mg/kg. These long term results in a cohort of acne patients treated with isotretinoin justify a reappraisal of the treatment strategy.

71P

Proteinic profile analysis in 10 patients with AIDS-related cryptosporidiosis.

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In patients with cryptosporidiosis and AIDS the humoral immunity don't have the most important role. Serum anti-*Cryptosporidium* immunoglobulins (Ig) have not a protective role. Total and *Cryptosporidium* fecal IgA, and intestinal IgA plasma cells increase, but this secretory immune response is uneffective. An alteration of interferon-γ secretion has been found in *lamina propria* of the colon.

Methods : The retrospective study of ten HIV-positive patients (9 men, 1 woman) with cryptosporidiosis allowed to analyse the proteinic profile : IGM, IgG, IgA, C3 complement, C-reactive protein (CRP), orosomucoid, haptoglobin, six months before the cryptosporidiosis (2 patients), at the diagnosis (10 patients), and 6 months after (8 patients).

Results and discussion : An increase of Ig was observed, which is very frequent in HIV-positive patients, who have an inadapted secretion of antibodies : IgM (4/10 patients) ; IgG (7/10) ; IgA (7/10). Conversely a decrease of Ig in relation to the gravity of the disease, cachexy and exsudative enteropathy, was observed too. CRP is increased in 6/10 patients, haptoglobin in 9/10 and orosomucoid in 10/10, because a chronic inflammatory syndrome.

The CRP is very variable with the current infections. The level of albumin and transferrin is decreased in 7/10 patients, in relation to their cachexy.

72P

SOWDA, A PARASITIC DERMATOSIS IN YEMEN. **D. RICHARD-LENOBLE, M. Thérizol-Ferly, T.H. Duong, A. Ferrer, Y. AL-Quabati and A. Alkholani - Department of Parasitology-Mycology, Tropical Medicine - Faculty of Medicine - 2 Bis Bd Tonnellé - 37032 Tours Cedex**

Following three investigations carried out in Yemen onchocerciasis focuses, a clinical, parasitological and serological study allowed to show the importance of the SOWDA as a special clinical picture within a population of *Onchocerca volvulus* microfilaria asymptomatic carriers as observed in Africa. This clinical picture of an unilateral acute onchodermatitis associates papules, hyperpigmentation, cutaneous hypertrophy and superinfections lesions due to an intense pruritus and localized usually at the leg with a lymphatic nodule at the groin.

The unilateral attack of this parasitic dermatosis transmited by black flies is unexplained. It responds to ivermectine which is a microfilaricide used in onchocerciasis mass control in Africa. (OCP-APOC program). A national fight program against this filariosis is developing in Yemen. It is based on a systematic distribution of ivermectine at a single dose of 200 µg / kg, two or three times a year in *Onchocerca volvulus* areas transmission.

73P

TOLERANCE PROFILE OF MINERALS CONTAINED IN SUNSCREENS

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Chemical UV-filters cause frequent irritation of the skin, especially in patients with certain photodermatoses. One possible alternative to reduce cutaneous irritation is the use of non-penetrating mineral blockers. Here, we investigated the tolerance profile of a combination of 10,5 % titanium dioxide and 2,4 % zinc oxide in a w/o vehicle (SPF 50). For this purpose, the following maximization tests were performed : 1°) the repeated insult patch-test in 100 subjects, including 9 iterative patch applications (24-hour exposure, 3 times a week for 3 weeks) of the preparation, followed by a 14-day rest period and a challenge on a previously unexposed test site ; 2°) the phototoxicity test in 20 subjects, where the test site is immediately irradiated with UV-A after application of the preparation, then covered for 24 hours, and evaluated immediately, 24 hours, 48 hours and 1 week after the removal of the patch ; 3°) the photoallergy maximization test in 100 subjects, consisting of an induction period of 6 exposures (twice weekly for 3 weeks) to 3 MED of solar simulating irradiation of the test site where the preparation had previously been applied under occlusive condition for 24 hours, and a challenge period including an occlusive 24 hour application of the preparation on a previously unexposed area, followed by a UV-A irradiation, and a reading of this area 48 hours and 72 hours after the irradiation. In the three experiments, appropriate controls were performed. The results of the three tests showed no positive reaction in any of the volunteers. Taken together, these results indicate that minerals have no potential of dermal irritancy, sensitization, phototoxicity or photoallergenicity. This confirms the high safety profile of mineral sunscreens.

74P

EVALUATION OF THE ANTI-FREE RADICAL ACTIVITY OF FORMULATIONS BY USING A LIVING SKIN EXPLANT MODEL. L. Friteau, L. Peno-Mazzarino, D. Castelli, G. Ries. RoC S.A. Department of Technology's Research and Development , Colombes, France.

In vitro models used to evaluate the anti-free radical activity are not often compatible with cosmetic formulations. The aim of this study was to develop a living skin explant model allowing application of cosmetic formulations to evaluate their potential anti-free radical activity. Skin explants were treated by a topical application of the tested product (25µl) for 18 hours. After washing with phosphate buffer (PBS), skin explants were irradiated with UVA and/or UVB and the supernatants were collected to measure, by fluorescence spectroscopy, the thiobarbituric acid-reactive substance (TBARS), taken as an indicator of lipid peroxidation. Firstly, we have demonstrated that UVB irradiation of skin explants induces lipid peroxidation in a dose-dependent manner. Treatment of skin explant with formulations, containing D,L-a-tocopherol (Vit E) or other products with known anti-free radical activity, lead to an inhibition of the lipid peroxidation.The living skin explant model has the advantage to allow testing of topical formulations in taking into account not only their anti-free radical activities but also the penetration capacities of the actives ingredients which is a fundamental factor to evaluate the efficacy of a topical formulation. Furthermore, by contrast with the other *in vitro* models used (cells in monolayer, reconstituted skin), it allows to take into account damages induced both on dermal and epidermal cells and therefore to be closer to *in vivo* conditions.

75P

HISTOLOGICAL AND BIOCHIMICAL EVALUATION OF U.V. A AND U.V. B EFFECT ON SURVIVING HUMAN SKIN. S. Boisnic, M.C. Branchet-Gumila, J-Y. Béranger L. Benslama. Pitié-Salpétrière hospital - Groupe de Recherche et d'Evaluation en Dermatologie et Cosmétologie (Gredeco), - Paris, France.

The aim of this study was to assess an ex-vivo method on human normal skin in surviving to evaluate the dermal and epidermal modifications after UV A and UV B rays.

We used a skin model maintained in viable conditions by organ culturing : skin fragments (plastic surgery) were placed in inserts which were then positioned over hollow culture slides. Skins were irradiated every other day for 8 days at 1, 2, 4, 8, 12, 20 J/cm^2 UV A or 50, 200, 600, 2000, 4000, 8000 mJ/cm^2 U.V. B and analysed by histological and biochimical studies.

UV B irradiation evidenced biochemical and histological modifications at the epidermic level. Keratinocyte clarification with a tinctorial affinity decrease of nuclei and cytoplasm, sun burn cells and pigmentary discharging were observed. We demonstated a decrease of cell viability (MTT test) and TNFα (immuno-enzymatic assay). Alterations increased with the UV dose applied on the skin (establishment of scores).

UV A irradiation induced a partial and progressive destruction (quantitative morphometry analysis) of elastic fibers and collagen.

This alternative method could allow to assess the degree of protection provided by sun creams with regard to UV damage.

76P

THE USE OF EPISKIN, A RECONSTRUCTED EPIDERMIS, IN THE EVALUATION OF PROTECTIVE EFFECT OF SUNSCREENS AGAINST CHEMICAL PHOTOTOXICITY INDUCED BY UVA.

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Phototoxicity is a well known side effect of treatment with chlorpromazine. This adverse reaction is elicited through the UVA-induced generation of a stable toxic photoproduct. In the present study we have demonstrated a UVA-dependent decrease in the cellular viability following topical treatment of the reconstructed epidermis Episkin with a non cytotoxic dose of chlorpromazine. It was also of importance to demonstrate that this *in vitro* model can be used to evaluate the protective effect of sunscreen formulation against chemically induced phtototoxicity. Given the possibility to apply sunscreen formulations under conditions of use and to deliver UVA from a solar simulator, different commercialy available products were tested on chlorpromazine pre-treated Episkin model.

Two formula containing respectively Mexoryl SX-titanium dioxide and Mexoryl SX-Parsol 1789-Eusolex 6300-titanium dioxide were tested. Both formula were totally effective in protecting the epidermal cell viability compared to the bare epidermis or the excipient treated epidermis.When increasing the UVA dose from 50 to 70 J/cm^2, the cell viability of the non protected epidermis was largely lowered, whereas the Mexoryl SX-titanium dioxide formula was still providing a certain protection and the Mexoryl SX - Parsol 1789 - Eusolex 6300 - titanium dioxide formula remained totally effective.

We have demonstrated that recontructed skin or epidermis can constitute useful in vitro models to study the phototoxic potential of topically applied chemicals and to assess the efficacy of sunscreen formulations with very high UVA protection in the prevention of this adverse reaction.

77P

THE USE OF EPISKIN, A RECONSTRUCTED EPIDERMIS, IN THE EVALUATION OF PROTECTIVE EFFECT OF SUNSCREENS AGAINST CHEMICAL LIPOPEROXIDATION INDUCED BY UVA.

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One of the advantages of reconstructed skin or epidermis over cultures of keratinocytes in monolayer is the presence of a fully differenciated horny layer which allows the topical application of sunscreen formulations and the assessment of their protective properties

The cytotoxic effect induced by UVA (320-400 nm) was investigated using a reconstructed epidermis (Episkin) on the basis of lipoperoxide production, cell viability and interleukin 1α release. A preliminary study showed that there exists a linear relationship between the dose of UVA (0 to 100 J/cm^2) and the amount of lipoperoxides produced. Moreover, this production was found to be closely related to the cellular content in glutathion. Thus, when an inhibitor of the glutathion-synthase was used before UVA irradiation of Episkin, an increase of lipoperoxides concomittant to a decrease of cell viability and an increase of IL 1α release was found.

Prior to UVA irradiation, different sunscreen formula were applied on Episkin and evaluated for their protective effect against lipoperoxidation. A striking lipoperoxide production was induced by UVA (50 J/cm^2) when a formula containing TiO_2-Uvinul TI 50-Parsol MCX-Parsol 1789 was used. On the contrary, no raise in lipoperoxide was found when Episkin was protected by a formula containing Eusolex 6300-TiO_2-Parsol 1789-Mexoryl SX. Finally, a decreased in lipoperoxidation was observed only when using the TiO_2- Mexoryl SX containing formula as compared with the lipoperoxides produced by non protected Episkin.

We have demonstrated that recontructed skin or epidermis can constitute useful *in vitro* models to study the UVA-induced epidermal damages among which those related to oxidative stress and to evaluate the efficacy of sunscreen formulations in the prevention of these adverse reactions.

78P

COMPARATIVE STUDY OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND STEREOLOGICAL IMAGE ANALYSIS OF EU- AND PHEOMELANIN CONTENTS IN MELANOCYTIC CELLS. E. Donois*, V. del Marmol#, K. Wakamatsu¶, S. Ito¶, G. Ghanem# and J.-E. Surlève-Bazeille*. *Dept. Cell. Biol., Univ. Bordeaux I, France. #Lab. Oncol. Exp. Surg., Jules Bordet Inst., Belgium. ¶Fujita Health Univ., School of Health Sci., Japan.

The aim of the study was to compare two methods quantifying eu- and pheomelanins, pigments synthesised by melanocytes. One is based on the high performance liquid chromatography (HPLC) quantification of specific degradation products of each melanin type. The other requires image analysis, transmission electron microscopy (TEM) and stereology. This study was carried out in cultured human melanoma cells of different melanogenic activities. For each line, melanins were measured by HPLC and cells were fixed and embedded as pellets for TEM. Ultrathin sections were treated or not by the alkali dissolving method allowing the elimination of pheomelanin from sections. The obtained micrographs were digitised then analysed with our image analysis program permitting the estimation of 7 primary parameters used in stereology. Stereological formulae were used for estimating melanosomal maturation, intracellular melanin content and the number of melanised melanosomes (MMs) per cell and this, for total melanin, eumelanin or pheomelanin. The results obtained show a good correlation between both methods for total melanin and particularly when using the cytoplasmic volume density of melanin (r=0.93). Moreover, we report that the number of MMs per cell and not the melanosomal maturation, is responsible for the differences observed between the different cell lines in total melanin (quantified by HPLC). These results demonstrate the relevancy of the stereological method, but none of the melanisation parameters obtained with both methods are correlated in the case of eu- or pheomelanin alone. Several hypothesis are put forward and the recent improvements in the HPLC determination of eumelanin, the utilisation of more pigmented cells and a new validation of the alkali dissolving method in relevant animal models could help us to explain these differences.

79P

β3 INTEGRIN SUBUNIT EXPRESSION AND METASTATIC POTENTIAL OF HUMAN MALIGNANT MELANOCYTES ARE CONVERSELY CORRELATED. Frédéric Bérard, Jacques Portoukalian, Michel Terrier, Luc Thomas. Service de Dermatologie, Hôpital de l'Antiquaille and Laboratoire de Glycobiologie de la Progression Tumorale, Lyon, France.

It has been previously reported that increased expression of β3 integrin subunit by melanoma cells was correlated with their metastatic potential (1). It has also been reported that the expression of VLA4 (α4β1) by malignant melanocytes was coversely correlated with this potential (2). We analyzed by western-blot and flow cytometry the expression of α4, β1, and β3 integrin subunits in eight clones and variants of a model of metastatic melanoma obtained in our laboratory after several cycles of s.c. injection of a human melanoma cell line in immunosuppressed new born rats. In these eight clones and variants which potential to grow as liver metastases ranges from low to very high, we didn't find any expression of α4 by both methods. β1 subunit was moderately expressed in all melanoma cells (about 30%), but we didn't find any difference in the expression in different clones (low, high, or very high metastatic potential). β3 subunit was highly expressed by low metastatic potential cells (67%) but only expressed by 30% of very high metastatic malignant melanocytes. These results suggest that VLA4 and β3 do not play a consistent role in melanoma cells metastatic behaviour.

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2 - Quian F. & col. *Cell*. **77** : 335-347 (1994)

80P

MELANOMA SCINTIGRAPHY WITH BENZAMIDE DERIVATIVES JL. Baulieu, L. Vaillant, F. Baulieu, D. Guilloteau, L. Machet, G. Lorette Nuclear Medicine and Dermatology departments, CHU Tours, France

The aim of this work was to assess the faisability of the detection of melanoma localization including brain, by using benzamide derivatives radiolabeled with iodine 123. In a preliminary study in the mouse B16 melanoma model, two candidat radiotracers were selected on the basis of high tumor uptake: N-(1-ethyl-2-pyrrolidinyl) methoxy-2-hydroxy-3-iodo-6-methoxy-benzamide (IBZM) currently used for brain dopamine D2 receptor scintigraphy, and N-(2-diethylaminoethyl)4-iodo-benzamide (BZA).

After informed consent, 6 patients (4 females and 2 males, mean age: 60 ± 13 yrs, extrema: 42 - 75 yrs) with cutaneous (2), lymph nodes (3), liver (1), brain (1) melanoma localizations received a 150 MBq IBZM injection. Brain SPECT (Ceraspect* DSI) was performed 1 to 2 hours after injection and whole body scans (Helix* Elscint) were obtained 3 and 24 hours later. No adverse effect to IBZM was noted. In all cases striatal uptake was achieved (striatum/cerebellum = 2.98 ± .34) and early uptake into 2 of 3 metastatic lymph nodes was observed. No uptake could be detected in the other known localizations.

In conclusion, IBZM scintigraphy provides inconstant visualization of melanoma metastatic lymph nodes and seems to be inapropriate for metastases screening because a lack of sensitivity. The study is carried on by using BZA.

81P

EXPRESSION OF EGF, EGF RECEPTOR, AND TGFα IN CUTANEOUS T CELL LYMPHOMA. P. Courville, J. Wechsler, E. Thomine, B. Vergier, C. Barry-Beylot, M. Bagot, P. Joly. CHU de Rouen, CHU de Créteil, CHU de Bordeaux et le Groupe français d'étude des lymphomes cutanées (G.F.E.L.C.).

Background : Pseudoepitheliomatous hyperplasia (PEH) have been occasionaly reported in cutaneous T cell lymphoma CTCL, specialy in CD30+ lymphomas.

Objective : To evaluate by immunohistochemistry the expression of EGF, EGFr and TGFα in CTCL with or whithout PEH.

Methods : Immunohistochemical staining with anti-EGF (Sigma), Anti-EGFR1 (Dako), Anti-TGFα, (Oncogene Science) was evaluated in 6 cases of CTCL with PEH, and compared to 5 cases of CTCL without PEH. Normal human skin and squamous cell carcinomas were used as controls. Formalin and frozen tissue sections were tested.

Results : Epidermal expression of EGF, EGFr, and TGFα was stronger in CTCL than that observed in normal human skin. Surprisingly, lymphomatous cells from CTCL with or without PEH expressed EGF and TGFα. No difference in the epidermal expression of EGF and TGFα could be evidenced between CTCL with and without PEH. In the contrary, the expression of the EGFr by the keratinocytes from CTCL with PEH was stronger than that of CTCL without PEH.

Conclusion : Our results suggest that lymphomatous cells of CTCL produce EGF and TGFα. An EGFr hyperexpression by the overlying epidermis may explain the PEH seen in some cases of CTCL.

82P

IMMUNOHISTOCHEMICAL STUDY OF THE EXPRESSION OF 3 DESMOSOMAL PROTEINS IN VARIOUS EPIDERMAL TUMORS. P. Courville, E. Thomine, P. Joly, F. Tron, P. Lauret, J. Hemet. Groupe de recherche en immunologie (GRIMP) France.

Background : Loss of expression of desmosomal proteins has been demonstrated in some carcinomas. These proteins, particularly cadherins seem to be involved in intercellular adhesion.

Objective : To evaluate the expression of 3 desmosomal proteins, in various epidermal tumors.

Methods :Ten squamous cell carcinomas (SCC), 4 basal cell carcinomas, 3 keratoacanthomas, 1 solar keratosis and 1 Bowen disease were studied by immunohistochemistry using the following monoclonal antibodies : Anti-desmoglein 1, anti-desmoplakins 1 and 2, and mAb F12 (a human mAb that recognized a 190-kD desmosomal plaque protein).

Results : The plasma membrane of the keratinocytes from human normal epidermis were strongly stained by the 3 mAbs. In the contrary, these 3 proteins were not expressed by SCC. In solar keratosis and Bowen disease, expression of these proteins was observed in areas with atypical keratinocytes. Interestingly, a low the 3 keratoacanthomas strongly expressed the 3 proteins. Basal cell carcinomas were particular because they demonstrated no or low expression of desmoglein 1, but strongly expressed intracellularly desmoplakins 1-2 and the protein recognized by mAb F12.

Conclusion : Desmosomal proteins seem to be differently expressed in various epidermal tumors. This could be useful to distinguish between SCC and keratoacanthoma.

83P

IMMUNOPHENOTYPING AND MORPHOLOGIC STUDY OF A NEW LEUKAEMIC CELL WITH CUTANEOTROPISM. D. Lipsker, B. Cribier, E. Heid, E. Grosshans. Clinique Dermatologique des Hôpitaux Universitaires de Strasbourg, FRANCE.

A new leukaemic cell, with a previously unreported phenotype, is presented. This cell was cutaneotropic, since the leukaemia occurred in a patient in whom specific cutaneous findings preceded for 10 months overt leukaemia.

A 81 years old patient had numerous bruiselike, infiltrated plaques and nodules and an inguinal lymphadenopathy. Biopsies of skin and lymphadenopathy showed a dense infiltrate of great cells, with a large irregular nucleus, which stained positive for CD4 and CD68, but negative for CD1, CD3, CD8, CD19, CD20, CD30, CD34 and CD57. Alpha-naphtyl-chloracetate esterase was negative. No extracutaneous involvement could be evidenced. Partial remission was obtained with chemotherapy. Ten months after the initial cutaneous lesions, she complained for the first time of fatigue and weakness and at this time overt leukaemia declared. Flow cytometry immunophenotyping (FACS) and electron microscopy (EM) of the peripheral blasts were carried out.

The FACS of the peripheral blasts showed that these cells were CD4, CD36, CD37, CD38, CD56, CD71 and HLA DR positive. They were not stem cells (CD34 negative) nor T cells (cytoplasmic CD3 negative) or B cells (cytoplasmic CD22 negative). EM showed monocytoid cells with perinuclear electron dense deposits.

The leukaemic cells of our patient expressed an incomplete natural killer (CD56 but not CD57) and monocytic phenotype (CD36, but not CD13, CD14, CD33). These cells had a marked cutaneotropism. To the best of our knowledge, leukaemic cells expressing such a phenotype have not yet been reported.

84P

DETECTION OF HUMAN PAPILLOMAVIRUS (HPV) TYPE 1 AND TYPE 2 IN CUTANEOUS WARTS AND PRODUCTION OF RECOMBINANT L1 CAPSID PROTEINS. C. Dupuy[1], L. Machet[2], D. Mahé[1], A. Touzé[1], G. Lorette[2], and P. Coursaget[1]. Laboratoire de Virologie, UFR Sciences Pharmaceutiques (1) and Service de Dermatologie, Centre Hospitalier Universitaire (2), Tours, France.

The aim of this study was to produce HPV-1 and HPV-2 recombinant virus particles in view to developpe serological tests for the detection of type-specific anti-human papillomavirus antibodies. Human papillomavirus infections are induced by a wide range of virus types affecting squamous stratified epithelia. The most common disease occurs in children and young adults who develop benign cutaneous warts associated mainly with HPV type 1 (plantar warts) and type 2 (vulgaris warts). Specimens from 44 patients with lesions of different clinical types (verruca plantaris, vulgaris, plana) were examined for HPV-1 and HPV-2 DNA by polymerase chain reaction. Specific primers located in the E4 region of HPV-1 and HPV-2 genomes were used. HPV DNA type 1 was found in 11.5% of patients, HPV DNA type 2 in 25% wherease 20.5% were positives for both HPV-1 and HPV-2. The E4 positive samples were then PCR amplified using L1 primers of HPV-1 and HPV-2 in view to obtained the entire L1 sequence (respectively 1526 and 1533 pb). It was not possible to amplified the entire L1 sequence of HPV-1, wherease in two occasion, L1 of HPV-2 was amplified. One of the two HPV-2 L1 gene obtained was cloned and expressed in insect cells using a L1 recombinant baculovirus. L1 protein of HPV-2 was produced in large amount, but did not autoassembled into recombinant viral particles in contrary to that we have previously observed for the production of L1 corresponding to genital HPV type 16 and 45. Others samples are under investigation in view to clone and express the L1 protein from HPV-1 and others HPV-2 strains.

85P

ALLERGENICITY POTENTIAL OF OAT EXTRACTS IN ATOPIC DERMATITIS PATIENTS.

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Oat extracts are widely used in the treatment of atopic dermatitis for their moisturizing and anti-pruritic properties. However, it has been speculated that contact dermatitis to oat proteins prevails in patients with atopic dermatitis. The purpose of this work was then to study the allergenic potential to oat in these patients. 50 patients with a documented history of atopic dermatitis were included in the study. Another group of 50 non atopic patients with an known history of contact allergy to allergens unrelated to oat ingredients were also tested as control. In each patient, patch-tests (Finn Chambers®) were performed on the backs, with the following ingredients : 3 % crude oat extract diluted in petrolatum, a commercially available cream containing a 3 % oat extract, and the respective vehicles. Prick-tests were carried out in the same patients, with the following ingredients : 3 % crude oat extract diluted in a phenolated glycerol solution, Stallergen® containing 15 % oat extract, and the respective vehicles. Patch-test results were evaluated after 48 and 72 hours, according to the ICDRG criteria. Prick-test reading was performed after 20 minutes. With the patch-tests, one atopic dermatitis patient and one control patient had a slightly positive reaction to the crude oat extract. Similarly, 2 additionnal patients were found positive with the oat cream, i.e one in each group. By prick-testing, 3 patients who had patch-test negative developped a positive reaction to oat extracts (2 atopic patients with the crude oat extract, 1 control patient with Stallergen®). In conclusion, these results suggest that patients with atopic dermatitis are not particularly prone to sensitization to oat extracts.

86P

STUDY OF CGRP, SUBSTANCE P AND VIP IN ATOPIC DERMATITIS :

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CGRP, Substance P (SP) and VIP appear to be particulary interesting in atopic dermatitis (AD) by their role in immunomodulation, itching and sweating.

Materials and methods : We compared by immunohistochemical technique the expression of those neuropeptides (NP) among 10 patients with AD and 11 healthy controls, on the anatomic regions of back and forearm. We also studied the cutaneous synthesis of CGRP by in situ hybridization (ISH) using a non-radioactive probe.

Results : No anatomical regional variation of the NP levels was observed. No difference of staining was demonstrated between patients and controls for VIP and SP. CGRP staining was more intense in patients with AD, along with a greater epidermal penetration of CGRP fibers. This suggests enhanced connections between CGRP fibers and Langerhans cells or keratinocytes in AD. We could not obtain any staining by ISH.

Conclusion : The expression of CGRP in AD appears to be particulary interesting, and studies using immunohistochemical techniques and ultrastructural analysis are required to determine the relation between CGRP fibers and epidermal cells.

87P

INTERLEUKIN-10 IS PRODUCED BY HUMAN KERATINOCYTES THROUGH IgE RECEPTOR (FcεRII/CD23) STIMULATION.

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The synthesis of IL-10 by human keratinocytes has been controversial. UVA1 and UVB irradiation seems until now to be the only way to induce IL-10 production by human keratinocytes. CD23 (FcεRII/CD23) is an activation antigen that has been previously shown to be expressed on human keratinocytes upon IL-4 stimulation. CD23 ligation leads to the transcription of the inducible Nitric-Oxide Synthase gene (iNOS) and to the release of proinflammatory mediators (TNF-α, IL-6). Recent data suggested that CD23-stimulated human monocytes produced IL-10. We then investigated IL-10 synthesis upon CD23 stimulation on normal human keratinocytes. Keratinocytes were first preincubated with IL-4 for 48 h and CD23 expression was checked using immunostaining. CD23+ keratinocytes were then stimulated with an anti-CD23 mAb or with IgE/antiIgE immune complexes. After 48 h and 72 h incubation, IL-10 levels peaked at 120 pg/ml in culture supernatants. IL-10 mRNA was detectable after 18h of stimulation of CD23+ keratinocytes, both with RT-PCR and blot after PCR products transfer to a nylon membrane. In addition, neutralization of IL-10 with an anti-IL-10 mAb increased both in magnitude and duration TNF-α production. Taken together, these data indicate that that the engagement of the CD23 molecule at the cell surface of human keratinocytes induces the generation of the immunosuppressive cytokine IL-10, and that this generation regulates the production of the proinflammatory cytokine TNF-α, suggesting the presence of a regulatory cytokine network during skin inflammatory responses.

88P

KINETIC OF IMMUNOREGULATORY CYTOKINES PRODUCTION BY EOSINOPHILS DURING THEIR DIFFERENTIATION *IN VITRO*

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Recently, eosinophils were shown to produce immunoregulatory cytokines of type 1(IFNγ, IL-2) and type 2 (IL-4, IL-5, IL-10). We were interested to study the kinetic of cytokines production by eosinophils during their differentiation. Mononuclear cells from ombilical cord blood, containing the CD34+ hematopoïetic stem cells, were cultured in the presence of IL-3, GM-CSF, and IL-5 for 4 weeks. Every week, cells were analyzed by flow cytometry for detection of IFNγ, IL-2, IL-4, and IL-10. To follow the eosinophilic differentiation, we have used a monoclonal antibody specific for the eosinophil peroxidase (EPO). EPO positive cells were observed as soon as one week of culture, increasing until 3 weeks of culture when the differentiation reached a maximum (65% EPO+ cells). During the first week, 20% of EPO+ cells produced IL-4. The next weeks, 55% of the differentiated eosinophils produced IL-2, whereas the IL-4 production decreased. Stimulations by antigens or microenvironment are excluded by this system of culture, therefore the differentiated eosinophils could be considered as naïve cells. Our results suggest the existence of an immature precursor of eosinophil, producing exclusively IL-2, as it was described for naïve T cells (Thp lymphocytes). The detection of IL-4 during the first weeks could be due to the existence of a common precursor between eosinophils and basophils.

91P

THE MONOCLONAL GAMMOPATHY OF THE SCHNITZLER SYNDROME RECOGNIZES AN EPIDERMAL ANTIGEN.

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The Schnitzler syndrome associates chronic urticaria to a monoclonal IgM gammopathy. It's pathophysiology is completely unknown. A new case of this rare syndrome is presented here and the mechanism of the urticaria was adressed by mean of an cutaneous immunoblot.

A 67 year old man had a 6 year history of permanent, antihistamine-resistant urticaria, night sweats and reccurent episodes of fever up to 39°C. Histological examination of a biopsy from an urticarial lesion showed oedema and a perivascular infiltrate of neutrophils and eosinophils in the papillary dermis. Immunofluorescence studies were negative. Serum protein electrophoresis demonstrated a monoclonal IgM with kappa light chains on immunofixation. The total IgM was 5,4 g/l (normal 0,4 - 3,1). Skeletal X-rays were normal. Bone marrow aspirates and histology showed no abnormality. The patients serum was analysed for the presence of anti-skin autoantibobies by the mean of immunoblotting on epidermal extract.

The patients serum recognized an 280/290 kD antigen on immunoblot. It is his monoclonal gammopathy which is responsible for the recognition, since the deposited immunoglobulins reacted only with anti-IgM and anti-κ sera, but not with anti-IgG, anti-IgA or anti-λ sera. Immunoblots with the sera of 3 patients with Waldenström's disease but without urticaria and 2 sera of patients with chronic idiopathic urticaria were negative.

The pathophysiogiology of the urticaria of the Schnitzler syndrome is not yet understood. The monoclonal IgM component can possibly intervene since it specifically recognizes an epidermal antigen. In this way, it could stimulate the keratinocytes which in turn could trigger the urticaria by cytokine release.

93P

EAR MOUSE OEDEMA AS A SIMPLE MODEL FOR EVALUATING IN THE *IN VIVO* ACTIVITY OF RETINOIC ACID ANALOGUES - EFFECT OF RAR AGONISTS, RAR ANTAGONISTS AND RXR AGONISTS.

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It has been shown that the irritant effect of retinoids and their clinical activity are correlated. We describe an *in vivo* model which uses the irritant effect of retinoids as an indication of their biological activities. After a single topical application on the ear of Balb/c mouse, RAR agonists induce a dose dependent inflammatory reaction characterised by an enhancement of ear thickness associated with an increase in epidermal thickness and a dermal oedema. This retinoid mediated effect can conveniently be measured with a micrometer. The oedema appears 3 days after application and reaches a maximum on day 6. This kinetic is the same for all the active RAR agonists tested. In this model, the minimal active dose for all-trans retinoic acid applied topically in acetone is 0.025 % and the minimal active dose for arotinoid acid tested under the same condition is 0.0003 %. CD2665 (a RARβ,γ antagonist) or AGN193109 (a RAR α, β,γ antagonist) have no irritant effect when applied alone but after topical or oral administration they inhibit the activity of RAR agonists in a dose dependent manner. CD2809 (an RXR agonist) has no irritant effect but after administration by the topical route or the oral route, it enhances the RAR agonist response. The ear mouse oedema model is a simple, useful assay for evaluating *in vivo* the activity of RAR agonists, RAR antagonists or RXR agonists. Formulations containing retinoids can also be tested in this model.

94P

EXOGENOUS MECHANICAL STRAIN INCREASES COLLAGEN AND ADHESION MOLECULES SYNTHESIS IN YOUNG COLLAGEN LATTICES

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The structural organization and synthesis of human dermis may be modulated by the intensity of mechanical forces within the tissue. Previous studies have shown the effect of an endogenous mechanical strain on collagen mRNAs levels and α2β1 integrin and fibronectin (FN) expression. To study the influence of an exogenous mechanical stress on the production for the same components, rectangular tense collagen lattices were specially created. They were obtained by mixing human fibroblasts (8 10^5C/ml) with bovine type I collagen (3mg/ml). On young lattices (24h) and on mature lattices (5d), 0.5% progressive extensions were made at a 5%/s rate for 20h. Collagen α1I and collagenase mRNAs were measured by RT-PCR, α2β1 integrin and FN expression were analysed by flow cytometry. At day 1, collagen synthesis was significantly increased (+289%, n=3) in « stressed » lattices as compared to « unstressed » lattices, this is no more observed at day 5. Collagenase was inversely regulated and was reduced (-50%) by day 1 and by day 5. In the same way, at day 1, α2β1 subunits and FN expression were significantly increased in « stressed » lattices. At day 5, the increase was much smaller.

We have demonstrated that mechanical extension can modulate cell phenotype. The sensitivity to mechanical stress seems to be in accordance with the age of lattice and its endogenous tension.

*Abstract nos. 89P, 90P, and 92P are unpublished.

95P

SOMATOSTATIN IS EXPRESSED IN NORMAL HUMAN EPIDERMIS.

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Somatostatin (SOM) consists in a fourteen or twenty-eight amino-acid neuropeptide. These two forms are biologically actives and are present in various tissue : brain, pancreas, SOM-28 is preominent in intestine. We have searched for the expression of SOM in human normal skin using different techniques : immunofluorescence (IF), confocal laser scanning microscopy (CLSM), radioimmunoassay (RIA), and chromatography.

Immunofluorescence study was performed using a rabbit polyclonal antiserum anti-SOM (Amersham). Double stanings with the same antibody as below and specific Langerhans cell (LC) monoclonal antibody directed to CD1a were observed in CLSM. RIA was performed with an antiserum 56D on extracts of human whole skin, or epidermal cell suspensions, or LC-enriched suspensions. These extracts were used for chromatography on SEPHADEX G50.

Suprabasal cells or whole epidermis were SOM-immunoreactive. CLSM showed that SOM positivity was expressed on the cytoplasmic membrane of LC. SOM concentrations in whole skin was 0,13±0,02 fmol/mg of tissue. SOM dosage in epidermal cell suspensions was 1,5±0,9 fmol/10^6 cells. Results for LC-enriched suspensions showed large variations among donors and anatomical site. Only one immunoreactive peak eluted like SOM-14 was observed on elution profil.

These results allow us to assert the presence of SOM-14 in human normal skin, and more especially in epidermis. This neuropeptide may be involved in skin biology and in pathophysiology of some skin diseases.

96P

IN VITRO MODULATION OF INTEGRINS ON BASAL KERATINOCYTES ($\alpha6\beta4$, $\alpha2\beta1$, $\alpha3\beta1$) BY INTERFERON-ALPHA AND INTERFERON-GAMMA.

L Tenaud, L. Sainte-Marie, O. Jumbou, P. Litoux, B. Dréno. Laboratory of cutaneous immuno-oncology C. H. U. Nantes France.

Interferon alpha (IFNα) and interferon gamma (IFNγ) stimulate auto-antibodies production directed against epidermal antigens. Thus, some studies have demonstrated some antibodies directed against basal membrane during treatments with INF-α and γ and in vitro upregulation of the Bullous Pemphigoid Antigen expression. It was shown that $\alpha_6\beta_4$ integrin and more particularly its α_6 subunit was tightly linked with Bullous Pemphigoid Antigens (180 and 220 kDa). The aim of our work is to study in vitro modulation induced by IFNα and IFNγ on the expression of $\alpha_6\beta_4$, $\alpha_2\beta_1$ and $\alpha_3\beta_1$ on normal keratinocytes. Normal human keratinocytes were cultured in monolayer, then incubated for 48 hours with IFN-α (1000 U/ml), IFN-γ (500 U/ml) and control medium. The integrins expression is evaluated by FACS. The experimentations were performed in duplicate.

Thus, we obtained an inhibitory effect of IFN-α on the expression of $\alpha2$, $\alpha3$, $\beta1$, $\alpha6$ and $\beta4$ and on the contrary an inductor effect of IFNγ on the expression of these subunits and also on $\alpha5$ and αV. In conclusion, IFN-γ and not α would be responsible for the in vitro up-regulation of $\alpha6\beta4$ expression. Present in large amount in pemphigoïd bullous blister fluid, it could be responsible for the up-regulation of these molecules expression and could maintain autoantibodies production.

97P

QUANTIFICATION OF SURFACE MOLECULES ON VIABLE HUMAN EPIDERMAL CELLS.

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In order to determine the absolute mean number of surface molecules per cell in human epidermis, we performed dual and triple color flow cytometric analysis of epidermal cell (EC) suspensions by using a quantitative immuno-fluorescence indirect assay with non-fluorescent plastic beads coated with different amounts of CD5 monoclonal antibodies. Beads standards were processed in parallel to EC to be tested and fixed in the same conditions. Fluorescence intensity measured in the different standards was used to calculate the standard regression line which allowed to convert mean fluorescence intensity values of surface markers into numbers of antigen molecules expressed per viable cell. For that purpose, we first demonstrated that Streptavidin-tricolor (SA-TC) was a reliable marker for non-viable and fixed EC. We verified that working concentrations of tested monoclonal antibodies were saturating. Our results demonstrated a weak expression of MHC class I molecules on viable $CD1a^+DR^+SA\text{-}TC^-$ epidermal Langerhans cells (LC) ($163 \pm 19 \times 10^3$ molecules/cell) compared to viable ($SA\text{-}TC^-$) keratinocytes ($785 \pm 110 \times 10^3$ molecules/cell) (n=5). Mean antigen density of HLA-DR and CD1a molecules on viable LC were $579 \pm 82 \times 10^3$ molecules/cell (n=5) and $1600 \pm 133 \times 10^3$ molecules/cell (n=5), respectively. Quantitative flow cytometry (i.e. quantimetry) on viable EC allows to evaluate the expression of membrane antigens and surface receptors on a cell per cell basis and may be of interest for group comparisons from multiple individuals. This new method may be proposed for monitoring therapeutic trials with immuno-phenotypic standardizing approaches.

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EVIDENCE THAT CORNEODESMOSIN IS A GLYCOSYLATED COMPONENT OF CORNEODESMOSOMES IN HUMAN EPIDERMIS.

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Recent data indicated the key role of corneodesmosomes (the corneocyte-specific intercellular junctions) in corneocyte cohesion and desquamation. In particular, proteolysis of one of their components, corneodesmosin, was described as essential for corneocyte detachment at skin surface. The heterogeneity of corneodesmosin migration in 2D gel electrophoresis, and the 2 potential N-glycosylation sites deduced from its sequence, recently obtained by cDNA cloning, suggest the protein to be glycosylated.

To test this hypothesis, proteins of a human epidermal extract containing the 52-56 kD corneodesmosin were immunodetected with G36-19 and F28-27, 2 monoclonal antibodies specific for corneodesmosin, before and after extensive treatment performed with various glycosydases: PNGase F, endo-α-N-acetylgalactosaminidase and neuraminidase. Glycoproteins of the extract were also affinity-purified by chromatography on ConA Sepharose and tested on immunoblots with both the antibodies. Finally, corneodesmosin was immunoprecipitated, transferred to membrane and analyzed with biotinylated lectins.

PNGase F treatment induced a decrease of roughly 5 kD in the apparent molecular mass of corneodesmosin. However, treatment with the other enzymes did not modify the gel migration of corneodesmosin. Moreover, the protein was eluted from ConA with methyl α-D-mannopyranoside, a competitive sugar.

These results show that human corneodesmosin is a glycoprotein comprising ~5 kD N-linked oligosaccharides, and suggest that sialic acid residues are not or slightly present in the molecule. The carbohydrate moieties may participate to putative adhesion properties of corneodesmosin and/or may transiently protect it against proteolysis, during maturation of the stratum corneum.

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MEASUREMENT OF β-GLUCOCEREBROSIDASE ACTIVITY *EX VIVO*

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A review of recent literature shows the stratum corneum to be the site of significant metabolic activity in terms of the initiation and maintenance of it's barrier function.

In terrestrial mammals, the phospholipids and glycolipids of the viable epidermis are replaced in the stratum corneum by a mixture of ceramides, free fatty acids and cholesterol which are localised in the extracellular spaces.

The arrangement of highly lipophilic ceramide and free fatty acid hydrocarbon chains confers to the stratum corneum it's cutaneous barrier function.

Among the enzymes responsable for these lipid conversions, β-glucocerebrosidase seems to have a fundamental role. For example, the topical treatment of mice with an inhibitor of this enzyme (bromoconduritol-β epoxide) leads to an acute disruption of barrier function.

We have developed a method for determining the glycolytic activity of β-glucocerebrosidase from strippings of human stratum corneum. This method has been applied to samples taken from a group of normal subjects to determine individual and seasonal variations of the hydrolytic activity of β-glucocerebrosidase.

For this, we used as substrate 4-methylumbelliferyl β D glucopyranoside, whose hydrolysis may be followed by the separation and quantification of the chromophore on silica plates.

This approach provides an additional, complementary insight for classical dermocosmetic evaluation methods with regard to skin barrier function physiology.

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STRATUM CORNEUM AND CUTANEOUS BARRIER. HISTOLOGICAL AND ULTRASTRUCTURAL STUDIES AFTER AN APPLICATION OF EMOLLIENT PREPARATION ON DESHYDRATED SKIN

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Considered before as an homogeneous protective layer, the stratum corneum after an ultrastructural examination is, in fact, an heterogeneous structure with a protein fraction : The corneocytes included in the lipid matrix. This organisation represents the skin barrier function. However, the stratum corneum water permeability is not only due to its lipid composition but also the structural arrangement of these intercorneocyte lipid layers. Besides, an acute lesion or /and a chronic irritation can alter this permeability by an epidermal superficial desorganisation. This alteration was realised by the dehydration by air of a skin biopsy, in experimental conditions. Then a restructural effect after an emollient substance application, was shown by various histological and ultrastructural studies.

An histological study, on cryosection slice after Mayer's hemalun staining, shows a stratum corneum thickness recovery. Nil Red penetration, assessed by confocal microscope, allowed to show a stain penetration decrease after a curative treatment with the emollient preparation comparatively to the damaged skin. A scanning electron microscopy observation of frozen specimen by simple cryogenic method allowed to observe the different epidermic layers and so a good cohesion of the stratum corneum after the treatment. An observation of some samples post fixed by Ruthenium tetroxyde, by transmission electron microscopy shows an amorphous matter in the first layers of stratum corneum. This emollient preparation (Ictyane) contributes to restructuration of the cutaneous barrier by diffusing through the stratum corneum interstices.

***Abstract no. 100P is unpublished.**